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NOTIFICATION OF ELECTION

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Applicant WUBBEN, Maria, Anna et al	

1. The designated Office is hereby notified of its election made:

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Title: Pectins as foam stabilizers for beverages having a foam head.

The invention relates to the use of pectins in the stabilization of foam heads of beverages such as beer.

In addition, the invention relates to methods for producing such pectins and beverages stabilized with such pectins.

Pectins are polysaccharides occurring in particular in the cell walls of dicotylous plants. The main chain of pectins contains α-D-galacturonic acid, while residues may contain L-rhamnose, D-galactose, L-arabinose, D-xylose and L-fucose. Each type of plant, in principle even each variety, possesses type-specific pectins whose compositions differ from those of the pectins of other types/varieties.

Hitherto, pectins have been used in particular in jelly-like products such as confiture and other fruit-jelly products. The pectins used herein are generally isolated from apple pulp and citrus pulp (see for instance US Patent Specification No. 4,943,443).

US Patent 5,008,254 describes pectins that are isolated from sugar beet pulp and can be used for improving various properties such as nutritional value and in many applications such as the improvement of consistency, non-hygroscopic adhesive, stabilizer of emulsions, etc.

In column 15 of the patent specification in question, the use of these pectins as a foam improver is mentioned, with the understanding that marshmallows and imitation whipped cream are involved here.

Of course, these permanent foams cannot be compared with the foam head of a beverage such as beer.

Beer differs from other beverages through, among other things, a persistent foam head.

Owing to the natural ingredients of beer and the specific know-how of the brewer, a foam of good quality can be obtained.

The most important properties of such a foam are:

- compactness
- slow, regular settlement
- good adhesion to the wall of the glass
- formation of fine-meshed "clings" during the drying of the foam.

These parameters, which are of particular importance for the consumer's appreciation of the beer, can be determined relatively objectively by means of equipment that is available on the market.

To obtain a high-quality foam, a foam stabilizer is added to various beers.

In general, the substance montol is used, although cobalt salts and iron salts are used as well.

In a number of countries, the addition of such substances is not allowed, as they are not necessary for the preparation of beer and/or are not inherent to beer.

Montol is a polypropylene glycol alginate (a composition of ß-D-mannuronic acid and α-L-guluronic acid having a molecular weight of between 30,000 and 200,000). This substance is isolated from algae. It is isolated in particular from the brown algae Laminaria digitata and Macrocystis pyrifera.

A known drawback of the use of montol, apart from the fact that it is not inherent in beer, are the chances of precipitate formation in the final product.

The invention provides a method for improving the stability of the foam head of beverages, wherein one or more pectins are added to the beverage before, during or after the process of its preparation.

Preferably added are pectins that have been isolated or extracted from the hop plant or other necessary starting materials for beer, on account of the fact that these pectins are derived from an ingredient that is inherent in beer and

hence will not affect the taste properties, which could well be the case with commercially available pectins from, for instance, citrus fruits.

Although hops are added in the form of hop cones, pellets, hop concentrates or isomerized hop extract during the process of brewing beer, their presence does not result in the presence of pectins from the hops with a foam-stabilizing action in the eventual beer, as the process conditions of the brewing process (for instance the high temperature at neutral pH during wort boiling) lead to the breakdown of the pectins, for instance due to, inter alia, the ß-elimination reaction according to Albersheim (Albersheim et al., 1960) (the breaking of glycoside bonds next to carboxymethyl groups). Due to this breakdown, their foam-improving capacity is also lost.

Hence, US Patent Specification No. 3,099,563, which relates to foam stabilizers for beer, starting from residual products of the brewing process, cannot relate to pectins from hops or other beer ingredients. It is not clear which substances are in fact prepared with the method according to this patent specification.

According to the present invention, it is preferred to start from pectins isolated from fresh hop parts or from by-products of the hop extraction.

Preferably, the pectins according to the present invention are obtained from the hop cones or the bines of the hop plant. The pectins do not need be isolated to a high purity, although this is in fact preferred, in particular because of the possible presence of undesired substances that may have a negative effect on the taste, the color or the foam stability of the eventual end product, such as for instance polyphenols.

The action of the pectins according to the invention is probably based on the same principle as the action of montol. Pectins as well as alginates possess a charge in beer (as described by Benard et al. Ann. Fals. Exp. Chim., 1981), enabling them to start an interaction with beer proteins. This may lead to a more stable foam.

If this charge of the pectins is indeed relevant for the foam-stabilizing action thereof, it may be advantageous to subject the isolated pectins to a partial saponification/de-esterification reaction. The average normal degree of esterification of 70% can then be reduced to 40-50%.

In the above-referred publication by Benard et al., pectins that may be present are only mentioned as being interfering during a montol determination, and nothing is mentioned about any function of those pectins.

The pectins according to the invention can be added at any desired moment from about 10 minutes before the end of the wort boiling (this is not critical) to the end of the preparation process. In any case, they have to be added late enough to prevent the above-mentioned breakdown from taking place to a large extent. Preferably, the pectins are added before the bright beer filtration, because any precipitates that may be present can be removed by means of the filtration. When, during the brewing process, a step known as posthopping (adding a hop preparation at the end of the wort boiling) is applied, the pectin preparation can suitably be added to this hop preparation.

The amounts of pectin that have to be added in order to achieve the improved stability can readily be determined by a skilled person. They will depend on, inter alia, the purity of the pectin preparation and the type of beer to which the preparation is added. In general, the amount of preparation to be added will be between 0.5 and 20 g/hl, preferably around 3 g/hl.

In principle, the invention is applicable to all types of beer for which a foam head is desired. The invention is in particular suitable for use in for instance beer of the pilsner type. (A bottom-fermented gold-colored beer having a characteristic hopped taste.)

According to the invention, with the pectins isolated from hops a foam stability is obtained that is at least as good as the foam stability obtained with montol, without the drawbacks attached thereto, and when the purity of the pectins

is sufficiently high, even a surprisingly better foam stability is obtained.

The invention will be explained in and by the following examples.

EXAMPLE 1

INTRODUCTION

The stabilization of the foam with pectin is probably based on the fact that pectin has a charge in beer. As a consequence, it may form compounds in the surface of the foam films. Hops contain 1-3% (d.s.) pectin. Hence, the pectins were isolated from hops and compared with commercially available pectins from Quest International.

RESULTS

When pectins are added to beer, an improvement of the foam stability can indeed be observed after incubation for 2 days by shaking of the bottle. The foam figures are given in Table 1 (Nibem meter).

Table 1		foam stability (sec)		average	test minus contr.
Hop pectin	(1 g/hl)	276	273	274	39
	5 "	266	285	275	40
	10 "	282	269	275	40
Pectin	1 "	283	262	272	37
(vis 200)	5 "	283	305	289	54
	10 "	300	301	300	65
Pectin	1 "	275	271	273	38
(200816)	5 "	289	288	288	53
	10 "	286	304	300	65
Control	0 "	225	245	235	--

1. Pectin exhibits good foam-stabilizing properties in dosages of 5 g/hl in beer.
2. The foam-stabilizing properties of hop constituents are based not only on those of the bittering substances, but also on those of the pectins from hops.

METHOD

From a water extract of hop cones, pectins (1-3% d.s.) can be extracted according to the following method:

1. Incubate the extract with 0.3 N HCl at 70°C for 4 hours. Then centrifuge after the pH has been adjusted to 3 with Na_2CO_3.
2. Next, add $Al_2(SO_4)_3$ and adjust the pH to 4 with Na_2CO_3. Separate the precipitate by centrifugation.
3. Next, add $Al_2(SO_4)_3$ and adjust the pH to 4 with Na_2CO_3. Separate the precipitate by centrifugation.

The pectins were added to bottles of beer in dosages as indicated in Table 1. After this, the bottles were shaken slowly at room temperature for two days. Finally, at the service laboratory, the foam stabilities were determined in duplicate.

EXAMPLE 2

2.1 MATERIAL

Exploratory experiments were conducted with Northern Brewer A, B and C (Dutch hops). The experiments were repeated with four other varieties (German hops). Northern brewer A and B originate from the same location, Northern brewer C comes from another location.

Table 2

Variety of hop	Hop cones	Bines	Waste
Northern brewer A	X	X	-
Northern brewer B	X	X	-
Northern brewer C	X	x	-
Hersbrücker	X	X	X
Aroma perle	X	X	X
Northern brewer	X	-	X
Brewers gold	X	X	X

For comparison, the foam stabilization experiments were also conducted with commercial citrus pectin (DE 67%) and montol. For the foam experiments, reference pilsner beer was used.

2.2 METHODS

1) Pectin extraction

The separate parts of the hop plant (bines, cones, leaves and the waste) were extracted with water (acidified to pH 2) to isolate pectin. The procedure followed is set forth in annex 1.

2) Determination of the AUA content and degree of esterification

The purity of the isolated pectin fractions was determined by means of a titration/saponification/titration. JECFA: Compendium of food additive specifications, volume 2, Food and Agriculture Organization of the United Nations, Rome 1992, p 1055.
The content of AUA (anhydrogalacturonic acid) can thus be determined. Thus, the degree of esterification (DE) of the fractions was determined as well.

3) Determination of the foam influence of pectin

The purified pectin fractions were added to beer to determine the influence thereof on the foam stability. The procedure is described in annex 2.

2.3 RESULTS

2.3.1 Dutch hops

The Dutch hop plants were harvested at two points of time to investigate variation in the maturity of the plant (time 1 is the proper moment of harvesting; the hop cones have the required maturity (plant A); time 2 is approx. 3 weeks after the proper time of harvesting (the leaves, cones and bines are

withered (plants B and C)). Table 3 shows the yields of the extracted pectin fractions. The leaves of all plants gave too low a yield of pectin, as a consequence of which they are not further considered separately.

Table 3 Yields of extraction from the Dutch hop plants

Pectin	weighed-in (g)	volume extraction (ml)	pectin weight (mg)	% extracted
Bine A	20	100	440	2.2
Bine B	90	500	310	0.34
Bine C	610	2600	3500	0.57
Cones A	30	250	440	1.2
Cones B	75	600	520	0.69
Cones C	160	1300	1400	0.88

At the proper time of harvesting, more pectin can be isolated from the bines and cones than approx. 3 weeks after this time. Particularly the bines are sensitive to the time of harvesting (the extracted pectin content decreases by 75-85%). The purity of the fractions also decreases (% AUA from 80 to 70). The degree of esterification of the cones and bines remains equal in time and is 75% for both.

Fig. 1 shows the influence of the hop pectins on the foam stability of beer in comparison with commercial citrus pectin (DE 67%) and montol (in annex 3 the results are given in tables). For dosing the pectin fractions 100% purity was assumed. However, the AUA content of bine A and cone A is 80%, the AUA content of bines B and C and cones B and C is 70%. For the comparison with montol, this should be taken into account. This was not corrected for in the experiments with the Dutch

hops, but it was corrected for in the experiments with the German hops.

Up to a content of 5 g/hl, the foam-stabilizing action of bine/cone pectin (A and B) is equal to the action of montoi. At higher concentrations, the action of pectin lags behind when compared with montol (partly due to the 70-80% purity of the fractions). Bine/cone pectin extracted from plant C shows a different pattern. The cone pectin has a negative effect on the foam stability, while the bine pectin has a greater positive effect on the foam stability in comparison with bine pectin of plant A and B. It is possible that in the case of the cone pectin (plant C) more foam-negative components (such as polyphenols) have been extracted along.

2.3.2 German hops

From the bines, cones and the waste of four German hop varieties, pectin was extracted as well.
The yields are given in Table 4. In the experiments, the leaves are not considered on account of the low pectin yields in Dutch hop plants.
Waste is a mixture of bines, leaves and cones such as it is left in the field after harvesting.

Table 4 Yields of the pectin extraction from the German hop plants

Pectin	weighed-in (g)	volume extraction (ml)	pectin weight (mg)	% extracted
Bine 1	250	1250	2.64	1.06
Bine 2	250	1250	5.46	2.18
Bine 4	250	1250	4.99	2.00
Cones 1	165	1750	4.36	2.64
Cones 2	165	1750	3.26	1.98
Cones 3	165	1750	2.36	1.43
Cones 4	150	1750	3.01	2.00
Waste 1	250	1750	4.04	1.62
Waste 2	250	1750	3.75	1.50
Waste 3	250	1750	6.73	2.69
Waste 4	250	1750	4.78	1.91

From the German hops a greater pectin fraction is extracted than from the Dutch hops. However, the purity of these preparations is lower than for the Dutch hops. This is probably due to the fact that for the German hops more material was purified at the same time. The AUA contents are shown in Fig. 2 (in annex 6 the results are shown in tables).

The average purity of the fractions is around 60%. The degree of esterification of all isolated pectin fractions is around 70% (in annex 6 the results are shown in tables).

Figs 3-5 show the influence of the different hop pectin fractions on the foam stability of reference beer in comparison with commercial citrus pectin (DE 67%) and montol (in annex 4 the results are shown in tables). In these Figures, a purity of 60% for the pectin fractions was assumed.

The concentration of the montol added was therefore also reduced to 60% to enable a good comparison between the two.

Bine pectin, hop cone pectin and montol give an almost equal foam stability after being added to reference beer. At an addition of 3 g/hl the foam improvement is approx. 40 sec. At a dosage of 3 g/hl, waste pectin gives an average foam improvement of 35 sec. To all pectin fractions it applies that the stabilization is variety-dependent. If the dosage of the pectin fractions is adjusted, so that 1, 5 and 10 g AUA/hl is dosed, the foam stability is not proportionally increased (see Fig. 6, in annex 5 the results are shown in tables). The fractions are only 60% pure on average, the other 40% may also consist of foam-negative components. If the dosage of the pectin fractions is increased, more foam-negative components may end up in the beer as well. In order to reduce or eliminate this problem, the fractions must be purified more.

After the addition to water and beer it was investigated whether the isolated pectin fractions were detectable by means of the montol test. As a standard, mannuronic acid was included. Fig. 7 shows the chromatograms. This proves that according to this method, pectin is not detectable. The course of the standard beer is identical to that of standard beer to which hop pectin has been added.

2.4 CONCLUSIONS

Pectins can be extracted from the different parts of the hop plant (bines, cones). The pectin yield from leaves was too small for experiments. Sufficient pectin can, however, be extracted from the waste that is left behind in the field after harvesting. The purity (AUA content) of the pectin fractions proves to depend on the time of harvesting, the amount of material during purification and the hop variety. The average degree of esterification of the Dutch hop plants is 75% and of the German hop plants 70%. Little difference is discernible between bines, cones or the waste. After addition to pilsner reference beer, "bine" and "hop cone" pectin yield the best foam improvements and these results are comparable

with montol additions. An addition of 3 g pectin or montol per hl yields a foam improvement of approx. 40 sec.

Annex to Example 2

ANNEX 1 PECTIN EXTRACTION FROM HOPS

1 Grinding the separate hop parts (cones, leaves, bines and the waste) (priorly freezing with nitrogen).
2 Adding warm water acidifying with HCl to pH 2.
3 Maintaining for 2 hours at 80°C under constant agitation.
4 Filtering over cheese cloth.
5 Mixing the supernatant with alcohol 96% (1:2) without neutralizing.
6 Filtering over cheese cloth.
7 Washing out precipitate 2x with 60% alcohol.
Washing out precipitate 1x with 96% alcohol (with intermediate fine-grinding with the ultraturrax).
8 Filtering over cheese cloth.
9 Drying overnight in Petri dish.

ANNEX 2 DETERMINATION OF THE FOAM INFLUENCE OF PECTINS

1 Dried pectins were crushed and dissolved in 5 ml water under heating before being added to beer in the following concentrations: 3 mg/bottle (approx. 1 g/hl), 15 mg/bottle (approx. 5 g/hl), 30 mg/bottle (approx. 10 g/hl). For this purpose, the pectin fractions were assumed to be 100 % pure. An experiment was conducted wherein the addition was based on the actual purity of the pectin fractions.
2 The bottles were shaken (50 rpm) for 48 hours (Dutch hops) or 60 hours (German hops).
3 Measurement of the foam stability with the Nibem meter.

ANNEX 3 RESULTS OF FOAM STABILITY AFTER ADDITION OF PECTINS TO BEER (PECTINS ISOLATED FROM DUTCH HOPS) - addition based on 100% purity

	Content added (g/hl)	Actual content AUA (g/hl)	Foam stability (sec)	Test minus control (sec)
commercial pectin	1	1	273	16
29-9-93	5	5	276	19
control 257 sec	10	10	287	30
12-10-93	1	1	273	7
control 267 sec	5	5	285	18
	10	10	296	29
28-10-93	1	1	288	8
control 276 sec	5	5	313	37
	10	10	320	44
bine A	1	0.8	265	8
29-9-93	5	4	281	24
control 257 sec	10	8	285	28
28-10-93	1	0.8	275	-1
control 276 sec	5	4	308	32
	10	8	316	40
bine B	1	0.7	298	22
26-10-93	5	3.5	310	34
	10	7	320	44
bine C	1	0.7	276	9
12-10-93	5	3.5	305	38
control 267 sec	10	7	318	51
28-10-93	1	0.7	283	7
control 276 sec	5	3.5	328	52
	10	7	-	-
cones A	1	0.8	265	8
29-9-93	5	4	284	27
control 257 sec	10	8	284	27
28-10-93	1	0.8	265	-9
control 276 sec	5	4	312	36
	10	8	319	43
cones B	1	0.7	289	13
26-10-93	5	3.5	290	14
	10	7	293	17
cones C	1	0.7	238	-29
12-10-93	5	3.5	192	-75
control 267 sec	10	7	180	-87
28-10-93	1	0.7	247	-19
control 276 sec	5	3.5	215	-61
	10	7	192	-84
montol	1	1	297	21
28-10-93	5	5	314	38
control 276 sec	10	10	340	64

ANNEX 4 RESULTS OF FOAM STABILITY AFTER ADDITION OF PECTINS TO BEER (PECTINS ISOLATED FROM GERMAN HOPS) - addition based on 100% purity

Experiment 15-11-1993	Content added (g/hl)	Actual content AUA (g/hl)	Foam stability (sec)	Test minus control (sec)
control water	-	-	302	-
control water	-	-	306	-
commercial pectin	1	1	323	19
	5	5	337	33
	10	10	356	52
montol	0.6	0.6	314	10
	1	1	335	31
	3	3	346	42
	5	5	367	63
	6	6	368	64
	10	10	381	77
bine 1	1	0.66	319	15
	5	3.3	346	42
	10	6.6	354	50
bine 2	1	0.75	323	19
	5	3.75	343	39
	10	7.5	374	69
bine 4	1	0.64	320	16
	5	3.2	336	32
	10	6.4	468	64
cones 1	1	0.61	325	21
	5	3.05	351	47
	10	6.1	365	61
cones 2	1	0.65	316	12
	5	3.25	342	38
	10	6.5	366	62
cones 3	1	0.56	319	15
	5	2.8	346	42
	10	5.6	360	56
cones 4	1	0.6	313	9
	5	3	341	37
	10	6	359	55
waste 1	1	0.55	320	16
	5	2.75	-	-
	10	5.5	345	41
waste 2	1	0.56	320	16
	5	2.8	339	35
	10	5.6	351	47
waste 3	1	0.72	309	5
	5	3.8	334	30
	10	7.2	360	56
waste 4	1	0.65	314	10
	5	3.25	343	39
	10	6.5	352	46

ANNEX 5 RESULTS OF FOAM STABILITY AFTER ADDITION OF PECTINS TO BEER (PECTINS ISOLATED FROM GERMAN HOPS) - addition based on purity measured

Experiment 19-11-1993	Content added (g/hl)	Actual content AUA (g/hl)	Foam stability (sec)	Test minus control (sec)
control water			301	-
bine 1	5.2	1	322	21
	31	5	348	47
	51	10	359	58
bine 4	4.7	1	323	22
	23	5	341	40
	47	10	373	72
montol	1.8	1.8	311	10
	3	3	329	28
	9	9	343	42
	15	15	358	57
	18	18	369	68
	30	30	378	77

ANNEX 6 PURITY OF THE PECTIN FRACTIONS (AUA CONTENT) AND DEGREE OF ESTERIFICATION (DE) OF THE GERMAN HOP VARIETIES

Sample	AUA (mg)	AUA (%)	DE (%)
pectin commercial	285	95	69
bine 1	146	66	76
bine 2	227	75	70
bine 4	192	64	73
cone 1	183	61	69
cone 2	194	65	75
cone 3	164	56	72
cone 4	181	60	68
waste 1	164	55	75
waste 2	167	56	77
waste 3	225	72	70
waste 4	195	55	77

EXAMPLE 3

3.1 MATERIAL

Residues of the following hop extracts were used:

A Ethanol extract residues
B CO_2 extract residues
C CO_2 extract residues
D Hexane extract residues

For comparison, the foam-stabilization experiments were also conducted with commercial citrus pectin (DE 67%), montol and priorly purified pectin fractions from hop bines and hop cones (Example 2)

For the foam experiments reference beer was used.

3.2 METHODS

3.2.1) Pectin extraction

The ground residues were extracted with water (acidified to pH 2) to isolate pectin. The procedure followed is set forth in annex 1.

3.2.2) Determination of the foam influence of pectin

The purified pectin fractions were added to beer in order to determine the influence thereof on the foam stability. The procedure is described in annex 2.

3.3 RESULTS

From different hop suppliers residues were obtained that are left behind after the production of hop extracts. From these residues pectins were isolated. The yields are shown in Table 5. The yields of pectin from these residues are comparable with the yields from fresh material (cones and bines). Residues from CO_2 extracts were obtained from two suppliers and reveal different pectin yields. However, the extraction procedure for the two suppliers is not completely known and different hop varieties were used. Example 2 has shown that the variety influences the amount of pectin that can be isolated.

Table 5 Yield of pectin fractions purified from residues formed during the preparation of different hop extracts

Sample		Pectin yield (%)
residues ethanol extract	A	2.3
residues CO_2 extract	B	1.8
residues CO_2 extract	C	2.5
residues hexane extract	D	2.4

Fig. 8 shows the influence of the residual pectins on the foam stability of pilsner beer in comparison with commercial citrus pectin, montol and bine pectin (see Example 2). In annex 3 the results are given in tables. For dosing the pectin fractions 100% purity was assumed. However, the AUA content of the residual fractions will be lower (was not determined). For the comparison with montol, this should be taken into account.

The foam-stabilizing action of pectin from ethanol and hexane extract residues is not substantial. Beer to which these pectins were added exhibits a foam stabilization that is virtually equal to that of control beer. A positive effect can be observed after the addition of pectins from residues of CO_2 extracts. At an addition of 10 g pectin/hl, the foam improvement is 26 sec. The pectins from bine and cones (previous experiment) give an increase of 40 sec, however without corrections having been made for the purity of the fractions.

ANNEX 1 (to Example 3) **PECTIN EXTRACTION FROM HOPS**

1 Grinding the different extracts (priorly freezing with nitrogen).
2 Adding warm water (water:material ratio, see Table 4.1), acidifying with HCl to pH 2.
3 Maintaining for 2 hours at 80°C under constant agitation. Filtering over cheese cloth. Mixing the supernatant with alcohol 96% (1:1.5) without neutralizing.
4 Filtering over cheese cloth.
5 Washing out precipitate 3x with 96% alcohol.
6 Filtering over cheese cloth.
7 Drying overnight in Petri dish.

ANNEX 2 (to Example 3) **DETERMINATION OF THE FOAM INFLUENCE OF PECTINS**

1 Dried pectins were crushed and dissolved in 5 ml water under heating before being added to beer in the following concentrations: 15 mg/bottle (approx. 5 g/hl) and 30 mg/bottle (approx. 10 g/hl). For this purpose, the pectin fractions were assumed to be 100% pure.
2 The bottles were shaken (50 rpm) at room temperature for 60 hours.
3 Measurement of the foam stability with the Nibem meter.

ANNEX 3 FOAM STABILITY OF BEERS TO WHICH DIFFERENT PECTIN FRACTIONS WERE ADDED

(to Example 3)

Sample	Amount added g/hl	Foam stability sec	Increased stability sec
Control	--	280	--
Control water	--	300	--
Residues hexane	5	300	0
extract A	10	294	0
Residues CO_2	5	302	2
extract B	10	327	27
Residues CO_2	5	300	0
extract C	10	326	26
Residues ethanol	5	296	0
extract D	10	298	0
Montol	5	345	45
	10	361	61
Commercial	5	323	23
pectin	10	355	55
Bine 1	10	344	44
Cones 1	10	338	38

CLAIMS

1. A method for improving the stability of the foam head of beverages, wherein before, during or after the preparation process of the beverage one or more pectins are added thereto.

2. A method according to claim 1, wherein an extract of one or more pectins, obtained from hops is used.

3. A method according to claim 2, wherein the extract is obtained from bines and/or cones of the hop plant.

4. A method according to claims 1-3, wherein the beverage is beer.

5. A method according to claim 4, wherein pectins are added during the preparation process as from 30 minutes before the end of the wort boiling, in such a manner that no significant part of the foam-stabilizing action is lost through boiling.

6. A method according to claim 5, wherein pectins are addded before the bright beer filtration.

7. A method according to any one of claims 4-6, wherein between 0.5 and 30 g pectin per hectoliter beer is added.

8. A method according to claim 7, wherein approximately 3-10 g pectin per hectoliter beer is added.

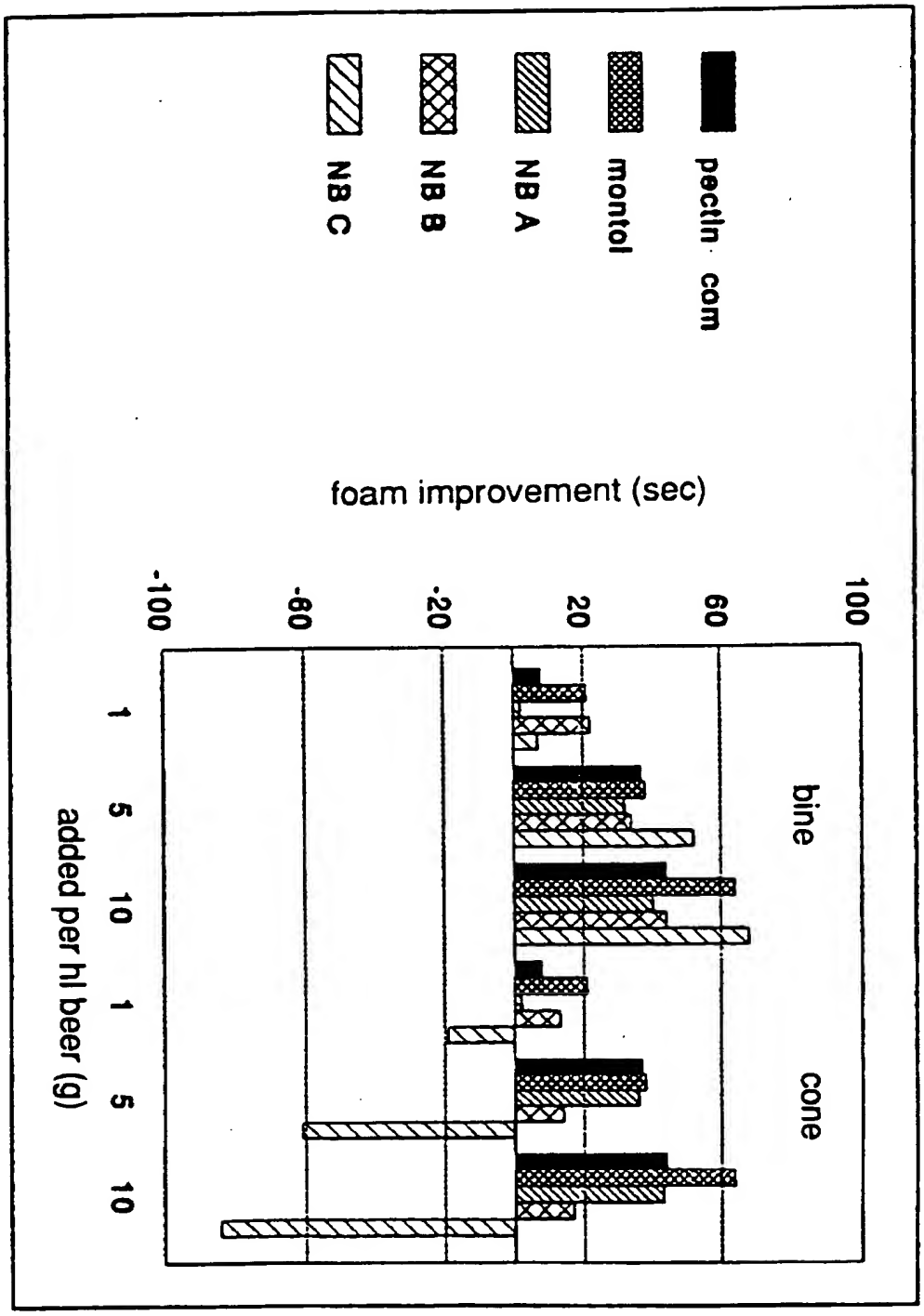
9. A beverage with a stabilized foam head, obtainable with a method according to any one of the preceding claims.

10. A beer with a stabilized foam head, obtainable with a method according to any one of claims 1-8.

11. A beer obtained according to any one of claims 1-8.

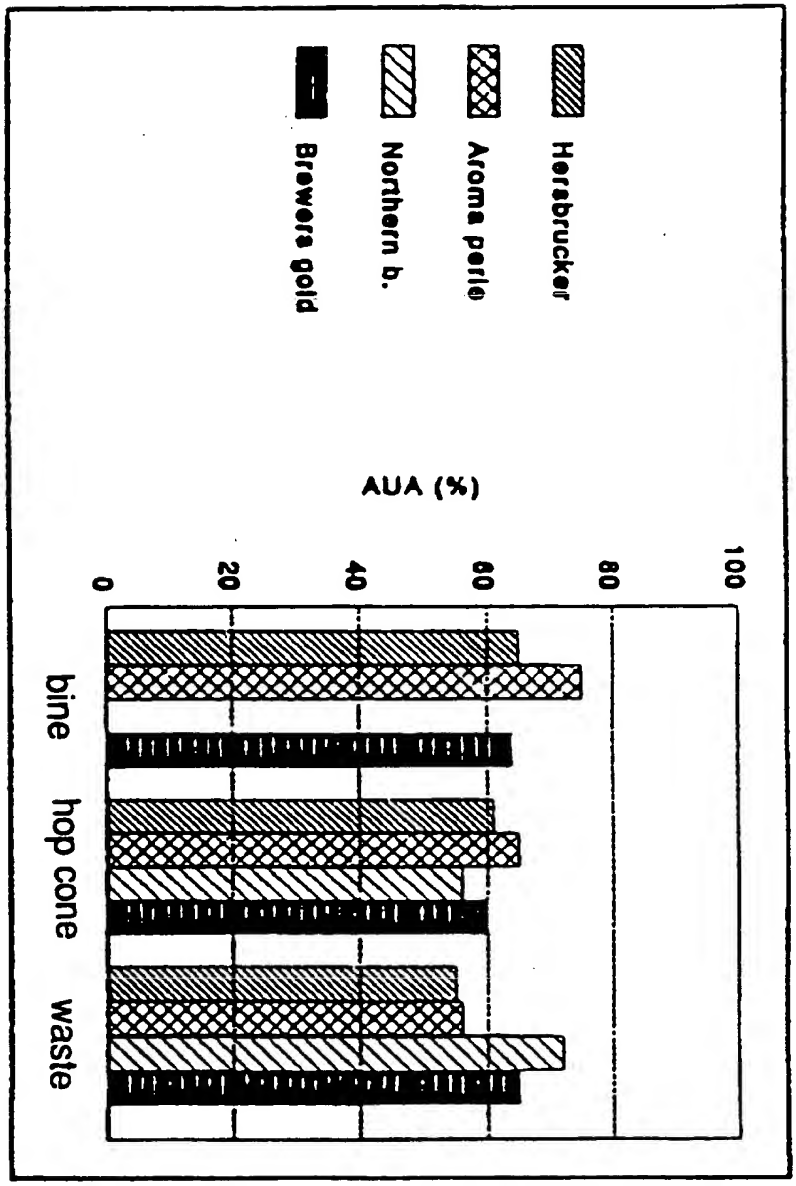
12. The use of hop pectins as foam stabilizer for foam heads of beverages.

13. A method for extracting pectins from hops, wherein hop plants or parts thereof are subjected to an extraction in an aqueous solution at a temperature of 50-100°C and a pH of 1-3.5.



Improvement of the foam stability of pilsner reference bear, after addition of hop pectin (from bines or cones), commercial pectin (100%) and montol (100%)

FIG.1



Purity (AUA contents) of the pectin fractions isolated from hops (bines, cones and waste)

FIG. 2

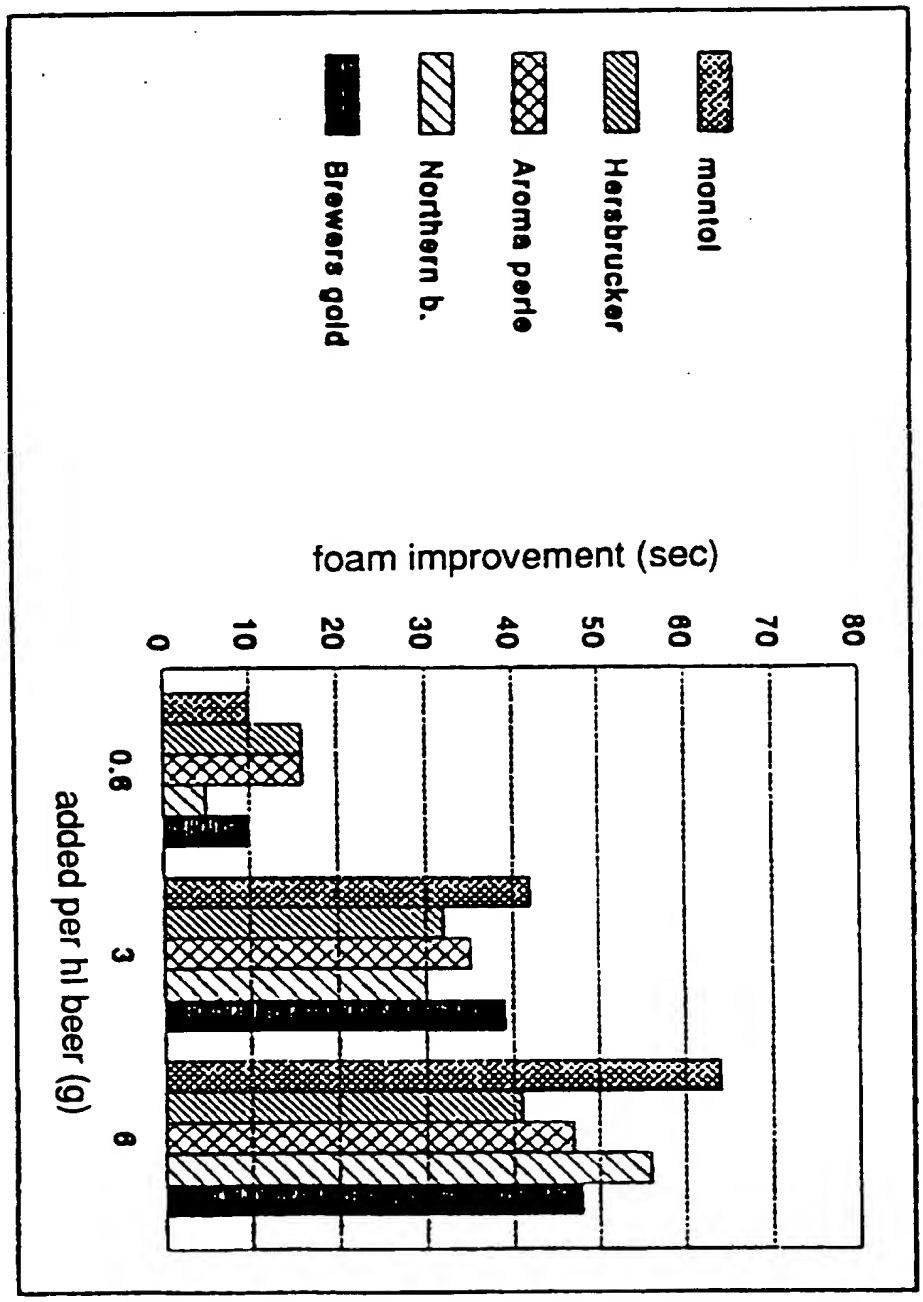

Improvement of the foam stability of pilsner reference bear, after addition of hop pectin from waste and montol (60%)
montol
Hersbrucker
Aroma perle
Northern b.
Brewers gold
foam improvement (sec)
0
10
20
30
40
50
60
70
80
0.6
3
6
added per hl beer (g)

FIG. 3

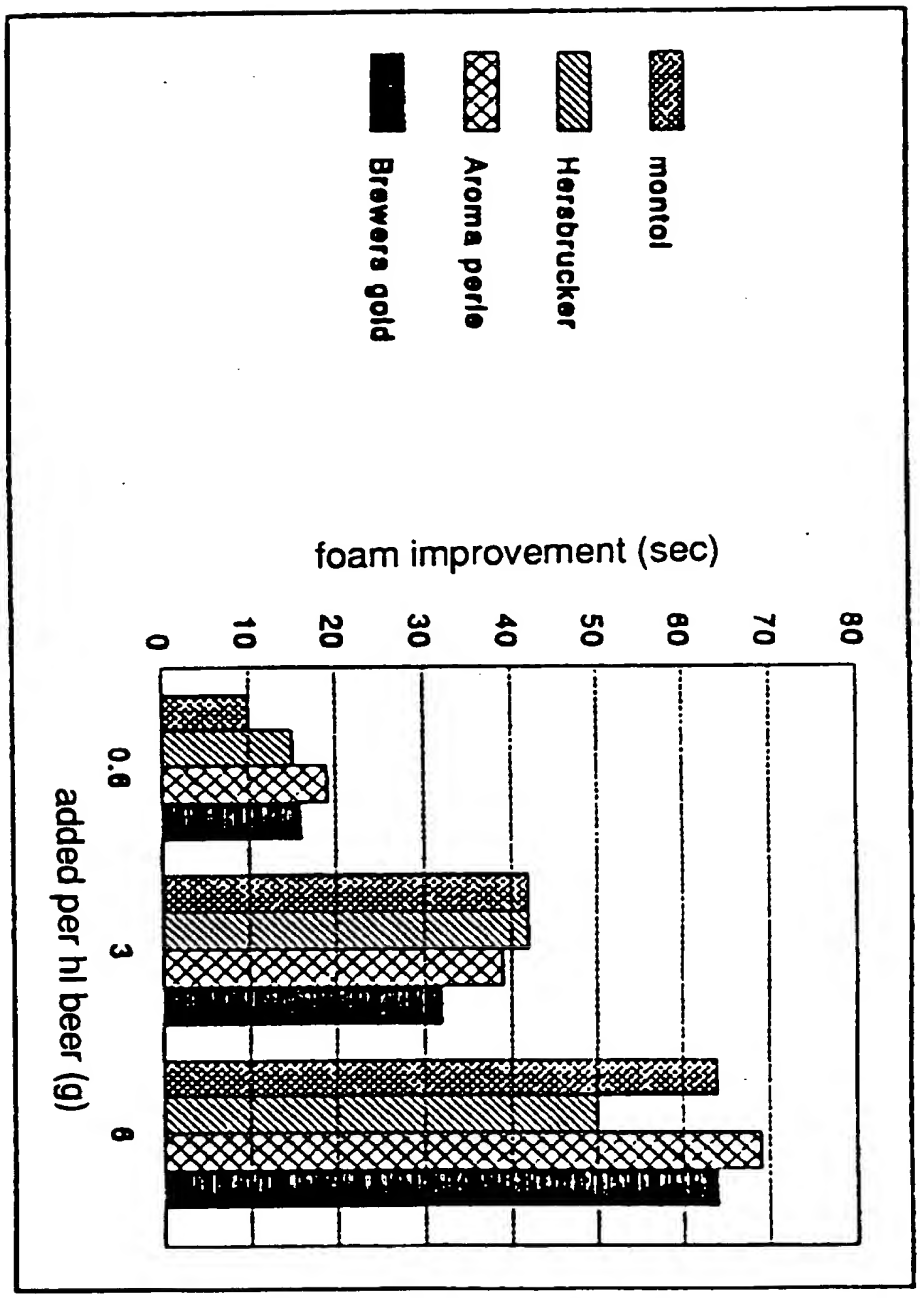

Improvement of the foam stability of pilsner reference bear, after addition of hop pectin from bines and montol (60%)
montol
Hersbrucker
Aroma perle
Brewers gold
foam improvement (sec)
0
10
20
30
40
50
60
70
80
0.6
3
6
added per hl beer (g)

FIG.4

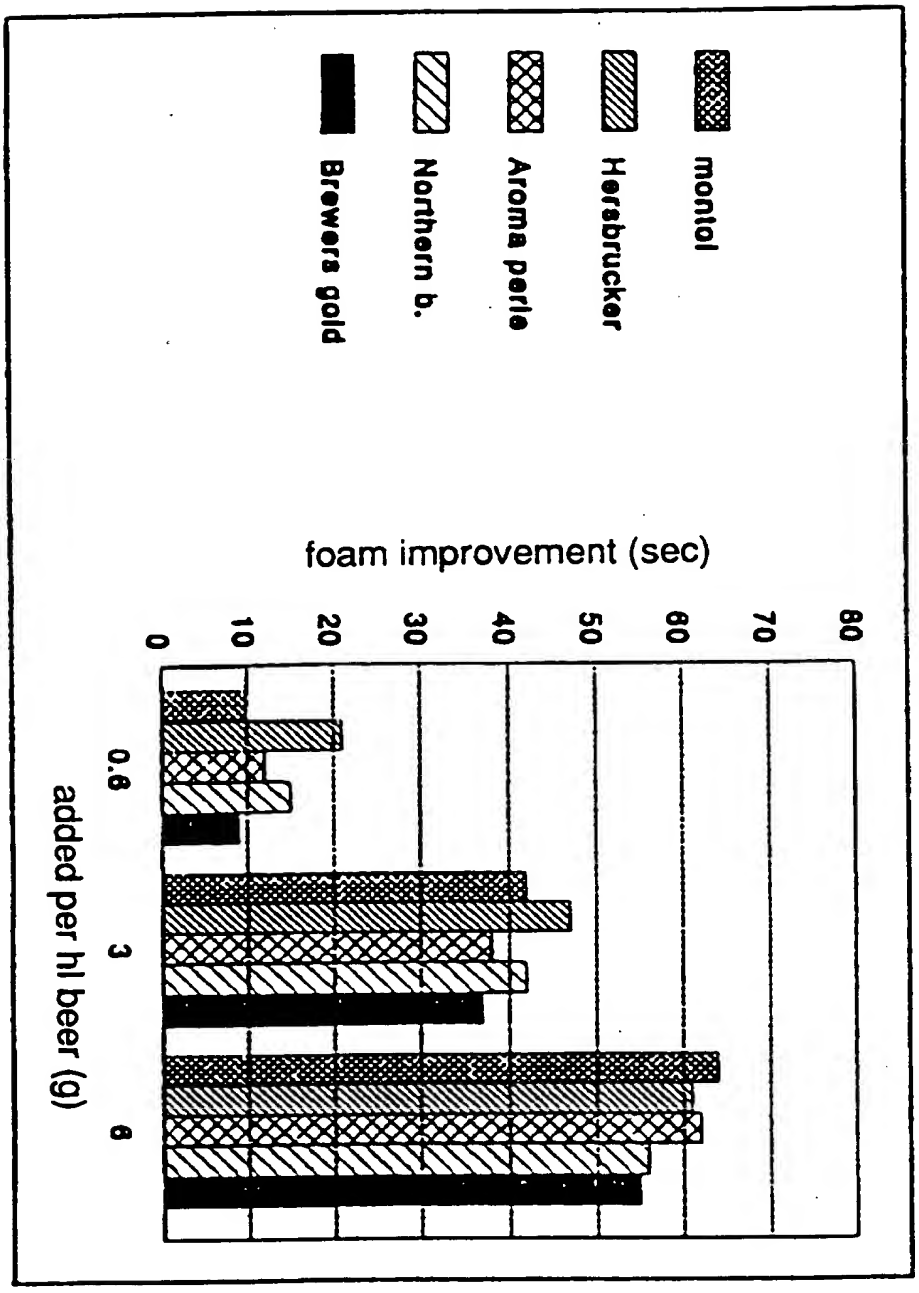

Improvement of the foam stability of pilsner reference bear, after addition of hop pectin from cones and montol (60%)
montol
Hersbrucker
Aroma perle
Northern b.
Brewers gold
foam improvement (sec)
0
10
20
30
40
50
60
70
80
0.6
3
6
added per hl beer (g)

FIG.5

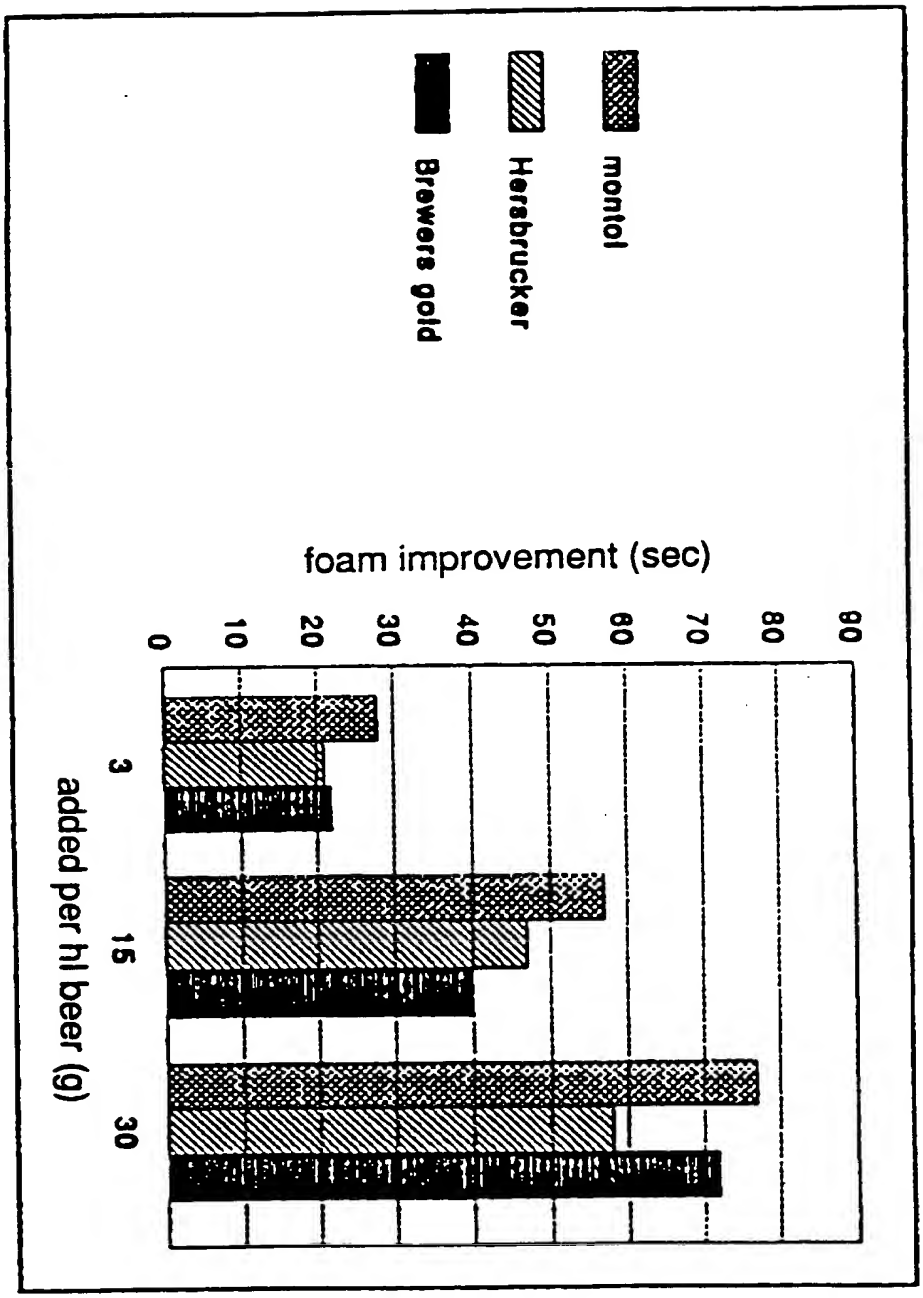

Improvement of the foam stability of pilsner reference bear, after addition of hop pectin from bines (corrected) and montol (100%)
montol
Hersbrucker
Brewers gold
foam improvement (sec)
0
10
20
30
40
50
60
70
80
90
3
15
30
added per hl beer (g)

FIG.6

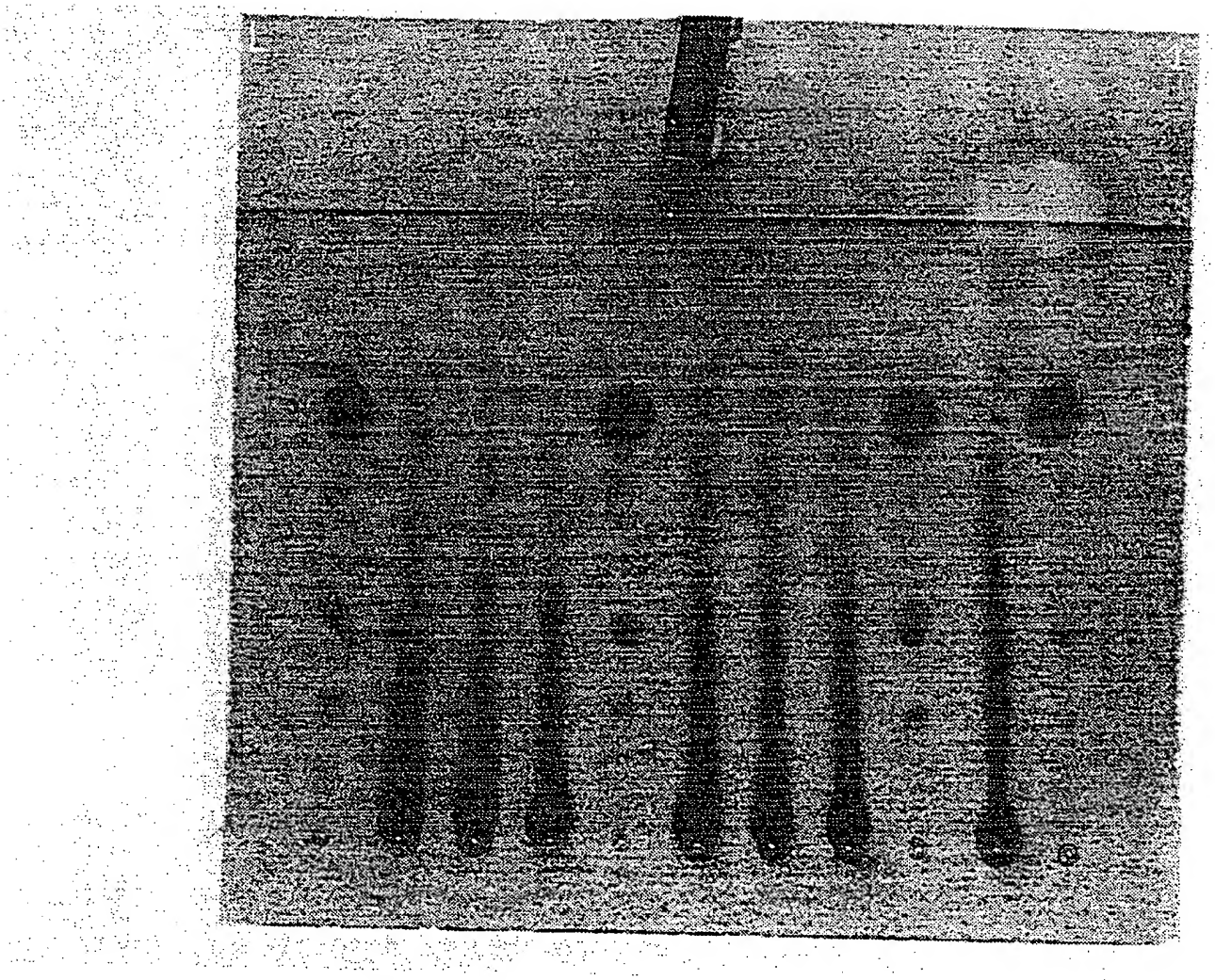


Fig. 7-1

Fig. 7-2

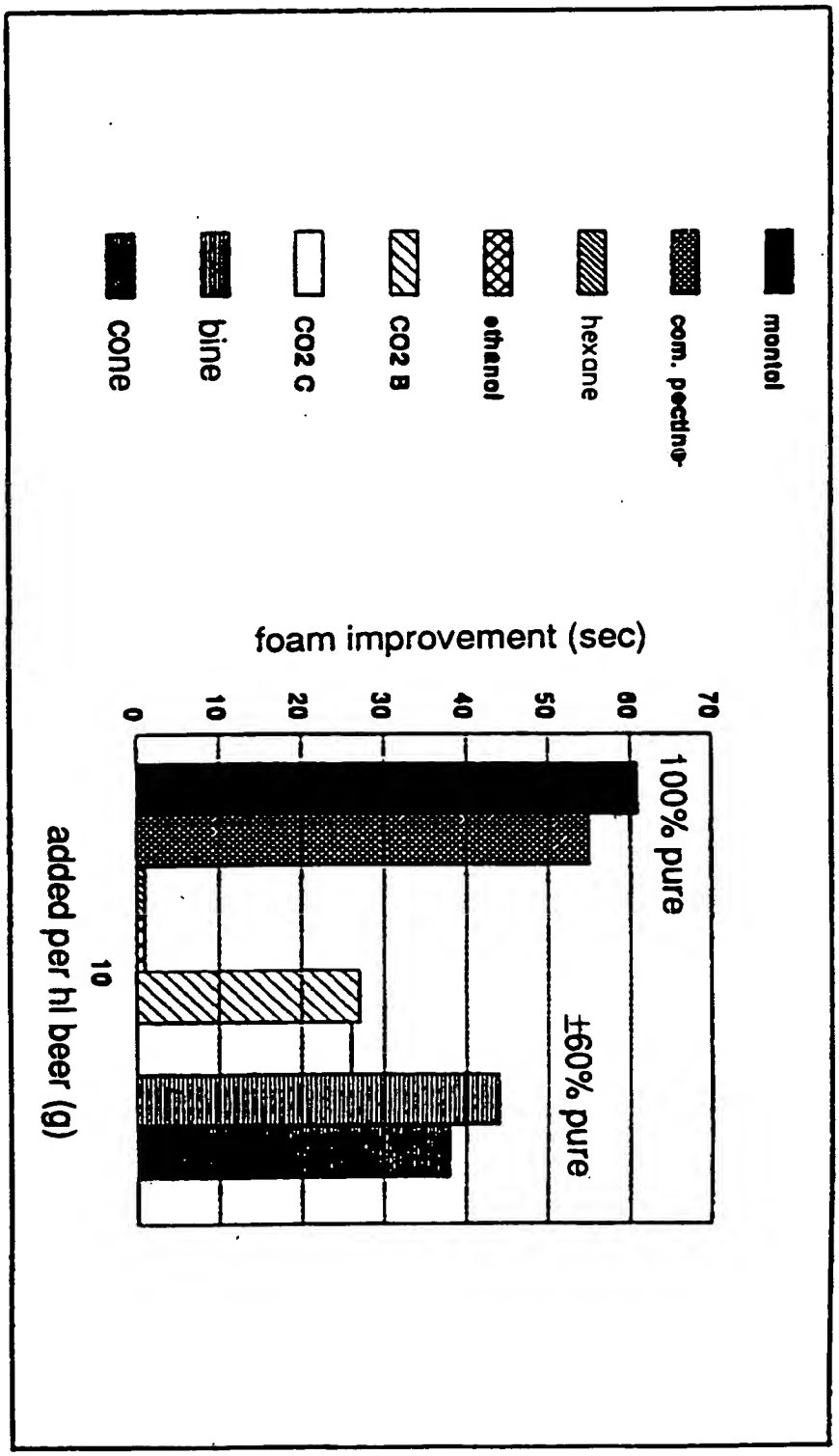


FIG.8

Improvement of the foam stability of pilsner reference bear, after addition of hop pectin from residues of hexane extracts, ethanol extracts and CO_2 extracts, montol (100%), bine pectin, hop cone pectin and commercial pectin (100%)

INTERNATIONAL SEARCH REPORT

Application No
PCT/NL 95/00266

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12H1/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12H C12C A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 292 034 (EVERS PAULUS HENDRICUS JOHANNN) 23 November 1988 see page 2, line 15 - line 17 ---	1,9,11
X	DATABASE FSTA INTERNATIONAL FOOD INFORMATION SERVICE (IFIS), FRANFURT/MAIN, DE AN 00188037, July 1980 SHKOP, YA. F. ET AL '(Method of producing beer.)' see abstract & SU,A,685 689 1979 ---	1,4,9-11
X	EP,A,0 426 434 (SBP, INC.) 8 May 1991 cited in the application see claims; example 2	1,9
A	---	11-13
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 October 1995	29.11.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Bevan, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Application No
PCT/NL 95/00266

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 463 696 (DE MELKINDUSTRIE VEGHEL B.V.) 2 January 1992 see the whole document	1,4,11
Y	GB,A,1 048 912 (M. BRIEM) 23 November 1966 see the whole document	1-13
Y	WO,A,93 15181 (RHONE-POULENC, INC.) 5 August 1993 see claims	1-13
A	DATABASE FSTA INTERNATIONAL FOOD INFORMATION SERVICE (IFIS), FRANFURT/MAIN, DE AN 00089682, December 1974 DROZDOVA, G. G. ET AL '(Pectic substances in barley and their role in beer and malt production.)' see abstract & IZVESTIYA VYSSHIKH UCHEBNYKH ZAVEDENII, PISHCHEVAYA TEKHNOLOGIYA, 1974, NO. 1, 21-25,	1,4,11
A	US,A,4 808 574 (I.I. BREKHMAN ET AL.) 28 February 1989 see column 2, line 13 - line 45	1,4,11
A	EP,A,0 243 654 (PEKTIN-FABRIK HERMANN HERBSTREITH KG) 4 November 1987 cited in the application see examples	1
A	US,A,3 099 563 (P.L.SMITH) 30 July 1963 cited in the application	
A	GB,A,1 082 284 (C. GORTATOWSKY) 6 September 1967	

INTERNATIONAL SEARCH REPORT

: Application No
PCT/NL 95/00266

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0292034	23-11-88	NL-A-	8700955	16-11-88
		CA-A-	1297820	24-03-92
		DE-A-	3872049	23-07-92
		US-A-	4943443	24-07-90
EP-A-426434	08-05-91	US-A-	5008254	16-04-91
		AU-B-	630755	05-11-92
		AU-B-	6564190	09-05-91
		CA-A-	2029022	02-05-91
		JP-A-	3197502	28-08-91
EP-A-463696	02-01-92	NL-A-	9001438	16-01-92
GB-A-1048912		NONE		
WO-A-9315181	05-08-93	AU-B-	3606393	01-09-93
		CA-A-	2127440	05-08-93
		CN-A-	1082104	16-02-94
		EP-A-	0625188	23-11-94
		FI-A-	943599	02-08-94
		NO-A-	942868	30-09-94
		US-A-	5387425	07-02-95
US-A-4808574	28-02-89	WO-A-	8911284	30-11-89
EP-A-243654	04-11-87	DE-C-	3614656	25-06-87
		AU-B-	7141487	05-11-87
		JP-A-	63039564	20-02-88
		ZA-A-	8703041	21-10-87
US-A-3099563	30-07-63	NONE		
GB-A-1082284		NONE		

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

RECORD COPY

For receiving Office use only

PCT/NL 95 / 00266
International Application No.

03 AUG. 1995 (03.08.95)
International Filing Date

BUREAU VOOR DE INDUSTRIËLE EIGENDOM
P.C.T. INTERNATIONAL APPLICATION
Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* PCT 0396

Box No. I TITLE OF INVENTION

Pectins as foam stabilizers for beverages having a foam head.

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Heineken Technical Services B.V.
2e Weteringplantsoen 21
1017 ZD Amsterdam
the Netherlands

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(i.e. country)* of nationality: NL

State *(i.e. country)* of residence: NL

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Wubben, Maria Anna
Arthur van Schendelplein 28
2624 CN Delft
the Netherlands

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: NL

State *(i.e. country)* of residence: NL

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Ir. Th.A.H.J. Smulders, ~~e.s.~~
~~c/o~~ VEREENIGDE OCTROOIBUREAUX
Nieuwe Parklaan 97
2587 BN The Hague
the Netherlands

Telephone No. 070 - 3500464

Facsimile No. 070 - 3522723

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

PCT/NL 95/00266

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Doderer, Albert
Albast 47
2719 TV Zoetermeer
the Netherlands

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: NL

State *(i.e. country)* of residence: NL

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
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- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- [X] AP **ARIPO Patent:** KE Kenya, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [X] EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- [X] OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [X] AM Armenia
- [X] AT Austria
- [X] AU Australia
- [X] BB Barbados
- [X] BG Bulgaria
- [X] BR Brazil
- [X] BY Belarus
- [X] CA Canada
- [X] CH and LI Switzerland and Liechtenstein
- [X] CN China
- [X] CZ Czech Republic
- [X] DE Germany
- [X] DK Denmark
- [X] EE Estonia
- [X] ES Spain
- [X] FI Finland
- [X] GB United Kingdom
- [X] GE Georgia
- [X] HU Hungary
- [X] IS Iceland
- [X] JP Japan
- [X] KE Kenya
- [X] KG Kyrgyzstan
- [X] KP Democratic People's Republic of Korea
- [X] KR Republic of Korea
- [X] KZ Kazakhstan
- [X] LK Sri Lanka
- [X] LR Liberia
- [X] LT Lithuania
- [X] LU Luxembourg
- [X] LV Latvia
- [X] MD Republic of Moldova
- [X] MG Madagascar
- [X] MN Mongolia
- [X] MW Malawi
- [X] MX Mexico
- [X] NO Norway
- [X] NZ New Zealand
- [X] PL Poland
- [X] PT Portugal
- [X] RO Romania
- [X] RU Russian Federation
- [X] SD Sudan
- [X] SE Sweden
- [X] SG Singapore
- [X] SI Slovenia
- [X] SK Slovakia
- [X] TJ Tajikistan
- [X] TM Turkmenistan
- [X] TT Trinidad and Tobago
- [X] UA Ukraine
- [X] UG Uganda
- [X] US United States of America
- [X] UZ Uzbekistan
- [X] VN Viet Nam

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- [ ]
- [ ]
- [ ]
- [ ]

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____.

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country *(in which, or for which, the application was filed)*	Filing Date *(day/month/year)*	Application No.	Office of filing *(only for regional or international application)*
item (1) NL	4 August 1994 (04. 08. 94)	9401273	
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☐ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s) : _____

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA /_____

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

Country (or regional Office):	Date *(day/month/year)*:	Number:
NL	3 May 1995	SN 24400 NL

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	:	4	sheets
2. description	:	21	sheets
3. claims	:	1	sheets
4. abstract	:	1	sheets
5. drawings	:	8	sheets
Total	:	35	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☒ priority document(s) *identified in Box No. VI as item(s):*
5. ☒ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☐ other *(specify)*:

Figure No. 1 of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

J. Renes

For receiving Office use only

1. Date of actual receipt of the purported international application: 03 AUG. 1995 (03. 08. 95)		2. Drawings: ☒ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:		
4. Date of timely receipt of the required corrections under PCT Article 11(2):		
5. International Searching Authority specified by the applicant: ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid	

For International Bureau use only

Date of receipt of the record copy by the International Bureau: 14 AUGUST 1995 (14. 08. 95)

Titel: Pectines als schuimstabilisatoren voor dranken met een schuimkraag.

De uitvinding heeft betrekking op de toepassing van pectines in de stabilisering van schuimkragen van dranken zoals bier.

Daarnaast heeft de uitvinding betrekking op werkwijzen voor het verkrijgen van dergelijke pectines en dranken gestabiliseerd met dergelijke pectines.

Pectines zijn polysacchariden die met name voorkomen in de celwanden van dicotyle planten. De hoofdketen van pectines bevat α-D-galacturonzuur, terwijl de residuen L-rhamnose, D-galactose, L-arabinose, D-xylose en L-fucose kunnen bevatten. Elke plantesoort, in principe zelfs elke variëteit bezit soort-eigen pectines die in samenstelling afwijken van de pectines van andere soorten/variëteiten.

Pectines vonden tot nog toe hun toepassing met name in gelei-achtige produkten zoals confiture en andere vruchtengelei-produkten. De hierin toegepaste pectines worden over het algemeen uit appel- en citruspulp geïsoleerd (zie bijvoorbeeld het Amerikaanse octrooischrift no. 4.943.443).

Het Amerikaanse octrooi no. 5.008.254 beschrijft pectines geïsoleerd uit suikerbietenpulp die te gebruiken zijn voor het verbeteren van allerlei eigenschappen zoals voedingswaarde en in velerlei toepassingen zoals als verbetering van consistentie, non-hygroscopisch kleefmiddel, stabilisator van emulsies, etc.

In kolom 15 van het betreffende octrooischrift wordt de toepassing van deze pectines als schuimverbeteraar gemeld, met dien verstande dat het hier om marshmallows en imitatieslagroom gaat.

Deze permanente schuimen zijn natuurlijk niet te vergelijken met de schuimkraag van een drank zoals bier.

Bier onderscheidt zich van andere dranken onder meer door een aanhoudende schuimkraag.

Dankzij de natuurlijke bestanddelen van bier en de specifieke kennis van de brouwer kan een schuim van goede kwaliteit verkregen worden.

De belangrijkste eigenschappen van een dergelijk schuim zijn.

- compactheid
- langzame, regelmatige inzakking
- goede adhesie aan de glaswand
- vorming van fijnmazige "clingen" bij opdrogen van het schuim

Deze parameters die van bijzonder belang zijn voor de appreciatie van het bier door de consument zijn redelijk objectief te bepalen met behulp van op de markt verkrijgbare apparatuur.

Aan diverse bieren wordt voor het verkrijgen van een kwalititatief hoogwaardig schuim een schuimstabilisator toegevoegd.

Over het algemeen wordt de stof montol toegepast, hoewel ook kobaltzouten en ijzerzouten gebruikt zijn.
In een aantal landen is de toevoeging van dergelijke stoffen niet toegestaan, aangezien ze niet noodzakelijk zijn voor de bereiding van bier en/of niet eigen zijn aan bier.

Montol is een polypropyleenglycol-alginaat (een samenstelling van ß-D-mannuronzuur en α-L-guluronzuur met een molecuulgewicht tussen 30.000 en 200.000). Deze stof wordt geïsoleerd uit algen. Het wordt met name geïsoleerd uit de bruine algen Laminaria digitata en Macrocystis pyrifera.
Een bekend nadeel van het gebruik van montol, afgezien van dat het niet bier-eigen is, is de kans op vorming van precipitaten in het eindprodukt.

De uitvinding voorziet nu in een werkwijze voor het verbeteren van de stabiliteit van de schuimkraag van dranken waarbij aan de drank voor, tijdens of na het bereidingsproces daarvan, één of meer pectines worden toegevoegd.

Bij voorkeur worden pectines toegevoegd die uit de hopplant of andere noodzakelijke biergrondstoffen zijn geïsoleerd of geëxtraheerd, vanwege het feit dat deze pectines uit een bier-eigen ingrediënt afkomstig zijn en daarom geen invloed zullen hebben op de smaakeigenschappen, wat bij commercieel verkrijgbare pectines uit bijvoorbeeld citrusvruchten wel het geval zal kunnen zijn.

Hoewel hop in de vorm van hopbellen, pellets, hopconcentraten of geïsomeriseerd hopextract wordt toegevoegd tijdens het proces van het bierbrouwen, leidt de aanwezigheid daarvan niet tot de aanwezigheid van pectines uit de hop met een schuimstabiliserende werking in het uiteindelijke bier, aangezien de procescondities van het brouwproces (bijvoorbeeld de hoge temperatuur bij neutrale pH bij het wortkoken) leiden tot afbraak van de pectines, onder andere door bijvoorbeeld de ß-eliminatie reactie volgens Albersheim (Albersheim et al., 1960) (het verbreken van glycoside bindingen naast carboxymethylgroepen). Ten gevolge van deze afbraak gaat ook hun schuimverbeterend vermogen verloren.

Het Amerikaanse octrooischrift no. 3.099.563 dat betrekking heeft op schuimstabilisatoren voor bier, waarbij uitgegaan wordt van restprodukten van het brouwproces kan daarom geen betrekking hebben op pectines uit hop of andere biergrondstoffen. Welke stoffen met de werkwijze volgens dit octrooischrift wel bereid worden, is niet duidelijk.

Volgens de onderhavige uitvinding wordt bij voorkeur uitgegaan van pectines geïsoleerd uit verse hopdelen of uit bijprodukten van de hop-extractie.

Bij voorkeur worden de pectines volgens de onderhavige uitvinding verkregen uit de hopbellen, danwel de stengels van de hopplant. De pectines hoeven niet tot hoge zuiverheid te worden geïsoleerd, hoewel dat wel de voorkeur verdient, met name vanwege de mogelijke aanwezigheid van ongewenste stoffen die de smaak, de kleur of de schuimstabiliteit van het uiteindelijke eindprodukt negatief kunnen beïnvloeden, zoals bijvoorbeeld polyfenolen.

De werking van de pectines volgens de uitvinding berust waarschijnlijk op hetzelfde principe als de werking van montol. Pectines zowel als alginaten bezitten een lading in bier (zoals beschreven door Benard et al. Ann. Fals. Exp. Chim., 1981), waardoor zij een interactie kunnen aangaan met biereiwitten. Dit kan leiden tot een stabieler schuim.

Wanneer deze lading van de pectines inderdaad van belang is voor de schuimsatabiliserende werking daarvan, kan het voordelig zijn om de geïsoleerde pectines te onderwerpen aan een gedeeltelijke verzepingsreaktie. De gemiddelde normale veresteringsgraad van 70% kan dan terug worden gebracht tot 40-50%.

In de gerefereerde publikatie van Benard et al. worden mogelijk aanwezige pectines alleen genoemd als storend bij een montolbepaling en wordt op enige functie van die pectines niet ingegaan.

De pectines volgens de uitvinding kunnen op elk gewenst moment vanaf ongeveer 10 minuten voor het einde van het wortkoken (dit is niet kritisch) tot aan het einde van het bereidingsproces worden toegevoegd. In ieder geval moeten zij zodanig laat worden toegevoegd dat de eerder genoemde afbraak niet in grote mate kan optreden. Bij voorkeur worden de pectines toegevoegd voor de helderbier filtratie, aangezien eventueel aanwezige precipitaten middels de filtratie kunnen worden verwijderd. Wanneer tijdens het brouwproces een stap wordt toegepast bekend als het nahoppen (het toevoegen van een hoppreparaat aan het eind van het wortkoken, kan aan dit hoppreparaat geschikt het pectinepreparaat worden toegevoegd.

De hoeveelheden pectine die toegevoegd moeten worden om de verbeterde stabiliteit te bewerkstelligen zijn door de vakman eenvoudig te bepalen. Zij zullen onder meer afhangen van de zuiverheid van het pectine-preparaat en de soort bier waaraan het preparaat wordt toegevoegd. Over het algemeen zal de hoeveelheid toe te dienen preparaat liggen tussen 0,5 en 20 g/hl, bij voorkeur rond de 3 g/hl.

De uitvinding is toepasbaar voor in principe alle biersoorten waar een schuimkraag gewenst is. Met name is de

uitvinding geschikt voor toepassing in bijvoorbeeld bier van het pilsner type. (Een laaggegist goudkleurig bier met een kenmerkende gehopte smaak.)

Volgens de uitvinding wordt met de pectines geïsoleerd uit hop een minstens even goede stabiliteit van het schuim verkregen als met montol, zonder de daaraan verbonden nadelen en wordt wanneer de zuiverheid van de pectines voldoende groot is zelfs een verrassend betere schuimstabiliteit verkregen.

De uitvinding zal worden toegelicht aan de hand van de volgende voorbeelden.

VOORBEELD 1

INLEIDING

De stabilisatie van het schuim met pectine berust waarschijnlijk op het feit dat pectine een lading heeft in bier. Hierdoor kan het wellicht verbindingen vormen in het oppervlak van de schuimfilmpjes. Hop bevat 1-3% (d.s.) aan pectine. Derhalve zijn de pectines uit hop geïsoleerd en vergeleken met commercieel verkrijgbare pectines van Quest International.

RESULTATEN

Wanneer pectines aan bier worden toegevoegd kan na 2 dagen incuberen onder schudden van de fles inderdaad een verbetering van de schuimstabiliteit worden opgemerkt. De schuimcijfers zijn weergegeven in tabel 1 (Nibem-meter).

Tabel 1		schuim-stabiliteit (sec)		gemiddeld	proef min blanco
Hoppectine	(1 g/hl)	276	273	274	39
	5 "	266	285	275	40
	10 "	282	269	275	40
Pectine	1 "	283	262	272	37
(vis 200)	5 "	283	305	289	54
	10 "	300	301	300	65
Pectine	1 "	275	271	273	38
(200816)	5 "	289	288	288	53
	10 "	286	304	300	65
Blanco	0 "	225	245	235	--

1 Pectine vertoont goede schuimstabiliserende eigenschappen in doseringen 5 g/HL in bier.

2. De schuimstabiliserende eigenschappen van hopbestanddelen berusten behalve op die van de bitterstoffen ook op die van de pectines uit hop.

METHODE

Uit een water-extract van hopbellen kunnen pectines (1-3% d.s.) geëxtraheerd worden volgens de volgende methode:

1. Extract incuberen met 0.3 N HCl bij 70°C gedurende 4 uur. Daarna centrifugeren, nadat de pH met Na_2C0_3 op 3 is gebracht.
2. Voeg vervolgens Al_2 $(S0_4)_3$ toe en stel de pH bij op 4 met Na_2C0_3. Centrifugeer het neerslag af.
3. Voeg vervolgens Al_2 $(S0_4)_3$ toe en stel de pH bij op 4 met Na_2C0_3. Centrifugeer het neerslag af.

De pectines zijn toegevoegd aan flessen bier in doseringen als aangegeven in tabel I. Hierna zijn de flessen twee dagen bij kamertemperatuur langzaam geschud. Tenslotte zijn op het servicelab de schuimstabiliteiten in duplo bepaald.

VOORBEELD 2

2.1 MATERIAAL

Met Northern Brewer A, B en C (Nederlandse hop) zijn oriënterende experimenten uitgevoerd. Met vier andere variëteiten (Duitse hop) zijn de experimenten herhaald. Northern brewer A en B zijn afkomstig van dezelfde locatie, Northern brewer C is van een andere locatie afkomstig.

Tabel 2

Variëteit hop	Hopbellen	Stengels	Afval
Northern brewer A	X	X	-
Northern brewer B	X	X	-
Northern brewer C	X	x	-
Hersbrücker	X	X	X
Aroma perle	X	X	X
Northern brewer	X	-	X
Brewers gold	X	X	X

De schuimstabilisatie experimenten zijn ter vergelijking ook uitgevoerd met commerciële citruspectine (DE 67%) en montol. Voor de schuimexperimenten is gebruik gemaakt van referentie pilsner bier.

2.2 METHODEN

1) Extractie van pectine

De afzonderlijke onderdelen van de hopplant (stengels, bellen, bladeren en het afval) zijn met water (aangezuurd tot pH 2) geëxtraheerd om pectine te isoleren. De gevolgde procedure is vermeld in bijlage 1.

2) Bepaling AUA-gehalte en veresteringsgraad

De zuiverheid van de geïsoleerde pectinefracties is bepaald met behulp van een titratie/verzeping/titratie. JECFA: Compendium of food additive specifications, volume 2, Food and Agriculture Organization of the United Nations Rome 1992, pp 1055.
Het gehalte AUA (anhydrogalacturonzuur) kan op deze manier bepaald worden. Hiermee is ook de veresteringsgraad (DE) van de fracties bepaald.

3) Bepaling schuiminvloed pectine

De gezuiverde pectinefracties zijn aan bier toegevoegd om de invloed ervan op de schuimstabiliteit te bepalen. De procedure staat beschreven in bijlage 2.

2.3 RESULTATEN

2.3.1 Nederlandse hop

De Nederlandse hopplanten zijn op twee tijdstippen geoogst om variatie te onderzoeken in de rijpheid van de plant (tijdstip 1 is het juiste moment van oogsten; de hopbellen hebben de vereiste rijpheid (plant A); tijdstip 2 is ± 3 weken na het juiste tijdstip van oogsten (de bladeren, bellen en stengels zijn hierbij verdord (plant B en C)). In tabel 3 zijn

de opbrengsten van de geëxtraheerde pectinefracties weergegeven. De bladeren van alle planten gaven een te lage opbrengst aan pectine, waardoor deze verder niet appart zijn meegenomen.

Tabel 3 Opbrengsten van extractie van de Nederlandse hopplanten

Pectine	ingewogen (g)	volume-extractie (ml)	pectine gewicht (mg)	% geëxtra-heerd
Stengel A	20	100	440	2,2
Stengel B	90	500	310	0,34
Stengel C	610	2600	3500	0,57
Bellen A	30	250	440	1,2
Bellen B	75	600	520	0,69
Bellen C	160	1300	1400	0,88

Uit de stengels en bellen is op het juiste moment van oogsten meer pectine te isoleren dan ± 3 weken na dit tijdstip. Vooral de stengels zijn gevoelig voor het tijdstip van oogsten (het geëxtraheerde pectinegehalte neemt 75-85 % af). Ook de zuiverheid van de fracties neemt af (% AUA van 80 naar 70). De veresteringsgraad van de bellen en stengels blijft in de tijd gelijk en bedraagt voor beide 75%.

In figuur 1 is de invloed van de hoppectines weergegeven op de schuimstabiliteit van bier in vergelijking met commercieel citruspectine (DE 67%) en montol (in bijlage 3 zijn de resultaten in tabellen weergegeven). Bij de dosering van de pectinefracties is uitgegaan van 100% zuiverheid. De AUA-gehalten van stengel A en bel A bedraagt echter 80%, van stengels B en C en bellen B en C bedraagt het AUA gehalte 70%. Bij de vergelijking met montol moet hier rekening mee gehouden

worden. Bij de experimenten met de Nederlandse hop is hiervoor niet en bij de experimenten met Duitse hop wel gecorrigeerd.

De schuimstabiliserende werking van stengel/bel pectine (A en B) is tot een gehalte van 5 g/hl gelijk aan de werking van montol. Bij hogere concentraties blijft de werking van hoppectine achter in vergelijking met montol (mede door 70-80% zuiverheid van de fracties). Stengel/bel pectine geëxtraheerd uit plant C laten een afwijkend patroon zien. Het bel pectine heeft hierbij een negatief effect op de schuimstabiliteit, terwijl het stengelpectine een groter positief effect heeft op de schuimstabiliteit in vergelijking met stengelpectine van plant A en B. Het is mogelijk dat in geval van het bel pectine (plant C) er meer schuimnegatieve componenten (zoals polyfenolen) zijn mee geëxtraheerd.

3.2 Duitse hop

Uit de stengels, bellen en het afval van vier Duitse hopvarieteiten is eveneens pectine geëxtraheerd.
De opbrengsten zijn weergegeven in tabel 4. De bladeren zijn in de experimenten niet meegenomen door de lage pectine opbrengsten bij Nederlandse hopplanten.
Afval is een mengsel van stengels, bladeren en bellen zoals dit na de oogst op het veld achterblijft.

Tabel 4 Opbrengsten van de pectine-extractie uit de Duitse hopplanten

Pectine	ingewogen (g)	volume-extractie (ml)	pectine gewicht (mg)	% geëxtra-heerd
Stengel 1	250	1250	2,64	1,06
Stengel 2	250	1250	5,46	2.18
Stengel 4	250	1250	4,99	2,00
Bellen 1	165	1750	4,36	2,64
Bellen 2	165	1750	3,26	1,98
Bellen 3	165	1750	2,36	1,43
Bellen 4	150	1750	3,01	2,00
Afval 1	250	1750	4,04	1,62
Afval 2	250	1750	3,75	1,50
Afval 3	250	1750	6,73	2,69
Afval 4	250	1750	4,78	1,91

Uit de Duitse hop wordt een grotere pectinefractie geëxtraheerd dan uit de Nederlandse hop. De zuiverheid van deze preparaten is echter lager dan bij de Nederlandse hop. Dit is waarschijnlijk te wijten aan het feit dat bij de Duitse hop meer materiaal tegelijk is opgewerkt. De AUA-gehalten zijn weergegeven in figuur 2 (in bijlage 6 zijn de resultaten in tabellen weergegeven).

De gemiddelde zuiverheid van de fracties ligt rond de 60%. De veresteringsgraad van alle geïsoleerde pectinefracties ligt rond de 70% (in bijlage 6 zijn de resultaten in tabellen weergegeven).

In figuur 3 t/m 5 is de invloed van de verschillende hoppectinefracties weergegeven op de schuimstabiliteit van referentie bier in vergelijking met commercieel citruspectine (DE 67%) en montol (in bijlage 4 zijn de resultaten in tabellen weergegeven). In deze figuren is ervan uitgegaan dat

de pectinefracties een zuiverheid van 60% hebben. De concentratie van het toegevoegde montol is daarom ook teruggebracht naar 60% om beide goed met elkaar te kunnen vergelijken.

Stengelpectine, hopbelpectine en montol geven een nagenoeg gelijke schuimstabiliteit na toevoeging aan referentiebier. Bij een toevoeging van 3 g/hl bedraagt de schuimverbetering ± 40 sec. Afvalpectine geeft bij een dosering van 3 g/hl gemiddeld een schuimverbetering van 35 sec. Voor alle pectine fracties geldt dat de stabilisatie variëteit afhankelijk is. Als de dosering van de pectinefracties wordt aangepast, zodat 1, 5 en 10 g AUA/hl wordt gedoseerd, wordt de schuimstabiliteit niet evenredig verhoogd (zie figuur 6, in bijlage 5 zijn de resultaten in tabellen weergegeven). De fracties zijn gemiddeld maar 60% zuiver, de overige 40% kan ook bestaan uit schuimnegatieve componenten. Als de dosering van de pectinefracties wordt verhoogd, kunnen er ook meer schuimnegatieve componenten in het bier terecht komen. Om dit probleem kleiner te maken of uit te sluiten moeten de fracties meer gezuiverd worden.

Na toevoeging aan water en bier is onderzocht of de geïsoleerde pectinefracties detecteerbaar waren met behulp van de montoltest. Als standaard is mannuronzuur meegenomen. In figuur 7 zijn de chromatogrammen weergegeven. Hieruit blijkt dat volgens deze methode pectine niet aantoonbaar is. Het verloop van het standaard bier is identiek als het verloop van standaardbier waaraan hoppectine is toegevoegd.

2.4 CONCLUSIES

Uit de verschillende onderdelen van de hopplant (stengels, bellen) zijn pectines te extraheren. De pectineopbrengst uit bladeren was te gering voor experimenten. Wel is er voldoende pectine te extraheren uit het afval wat na de oogst op het veld achterblijft. De zuiverheid (AUA-gehalte) van de pectinefracties blijkt af te hangen van het oogsttijdstip, hoeveelheid materiaal bij opwerken en de hopvarieteit. De veresteringsgraad van de Nederlandse hopplanten is gemiddeld

75% en van de Duitse hopplanten 70%. Er is hierbij weinig onderscheid zichtbaar tussen stengels, bellen of het afval. Na toevoeging aan pilsner referentie bier leveren "stengel" en "hopbel" pectine de beste schuimverbeteringen op en deze resultaten zijn vergelijkbaar met montoltoevoegingen. Een toevoeging van 3 g pectine of montol per hl levert een schuimverbetering van ± 40 sec op.

Bijlage bij voorbeeld 2

BIJLAGE 1 EXTRACTIEPECTINE UIT HOP

1 De afzonderlijke hoponderdelen (bellen, bladeren, stengels en het afval) malen (vooraf bevriezen met stikstof)
2 Warm water toevoegen (water:materiaal verhouding, zie tabel 4.1 en 4.2), aanzuren met HCl tot pH 2
3 Gedurende 2 uur bij 80°C onder constant roeren
4 Filtreren over kaasdoek
5 Supernatant mengen met alcohol 96% (1:2) zonder te neutraliseren
6 Filtreren over kaasdoek
7 Neerslag 2x uitwassen met 60% alcohol
Neerslag 1x uitwassen met 96% alcohol (tussendoor met de ultraturrax fijnmalen)
8 Over kaasdoek filtreren
9 Gedurende de nacht dogen in petrischaal

BIJLAGE 2 BEPALING SCHUIMINVLOED VAN PECTINEN

1 Gedroogde pectinen worden fijngemaakt en onder verwarming opgelost in 5 ml water alvorens ze worden toegevoegd aan bier in de volgende concentraties: 3 mg/flesje (± 1 g/hl), 15 mg/flesje (+ 5 g/hl), 30 mg/flesje (+ 10 g/hl). Hierbij is ervan uitgegaan dat de pectinefracties 100 % zuiver waren. Een experiment wordt uitgevoerd waarbij de toevoeging gebasseerd is op de werkelijke zuiverheid van de pectinefracties.
2 De flesjes worden geschud (50 rpm) gedurende 48 uur (Nederlandse hop) of 60 uur (Duitse hop)
3 Meting van de schuimstabiliteit met de Nibem-meter.

BIJLAGE 3 RESULTATEN SCHUIMSTABILITEIT NA TOEVOEGING VAN PECTINEN AAN BIER (PECTINEN GEISOLEERD UIT NEDERLANDSE HOP)
toevoeging gebasseerd op 100% zuiverheid

	gemeten toegevoegd (g/hl)	werkelijk gemeten AUA (g/hl)	schuimstabiliteit (sec)	proef min blanco (sec)
commercieel pectine	1	1	273	16
29-9-93	5	5	276	19
blanco 257 sec	10	10	287	30
12-10-93	1	1	273	7
blanco 267 sec	5	5	285	18
	10	10	296	29
28-10-93	1	1	288	8
blanco 276 sec	5	5	313	37
	10	10	320	44
stengel A	1	0.8	265	8
29-9-93	5	4	281	24
blanco 257 sec	10	8	285	28
28-10-93	1	0.8	275	-1
blanco 276 sec	5	4	308	32
	10	8	316	40
stengel B	1	0.7	298	22
26-10-93	5	3.5	310	34
	10	7	320	44
stengel C	1	0.7	276	9
12-10-93	5	3.5	305	38
blanco 267 sec	10	7	318	51
28-10-93	1	0.7	283	7
blanco 276 sec	5	3.5	328	52
	10	7	---	--
bellen A	1	0.8	265	8
29-9-93	5	4	284	27
blanco 257 sec	10	8	284	27
28-10-93	1	0.8	265	-9
blanco 276 sec	5	4	312	36
	10	8	319	43
bellen B	1	0.7	289	13
26-10-93	5	3.5	290	14
	10	7	293	17
bellen C	1	0.7	258	-19
12-10-93	5	3.5	192	-75
blanco 267 sec	10	7	180	-87
28-10-93	1	0.7	247	-19
blanco 276 sec	5	3.5	215	-61
	10	7	192	-84
noacol	1	1	297	21
28-10-93	5	5	314	38
blanco 276 sec	10	10	340	64

BIJLAGE 4 RESULTATEN SCHUIMSTABILITEIT NA TOEVOEGING VAN PECTINEN AAN BIER (PECTINEN GEISOLEERD UIT DUITSE HOP) - toevoeging gebasseerd op 100% zuiverheid

experiment (5-11-1993)	gehalte toegevoegd (g/hl)	werkelijk gehalte (UA g/hl)	schuimstabiliteit (sec)	proef min blanco (sec)
blanco water	-	-	302	--
blanco water	-	-	306	--
commercieel pectine	1	1	323	19
	5	5	337	33
	10	10	356	52
mentol	0.6	0.6	314	10
	1	1	335	31
	3	3	346	42
	5	5	367	63
	6	6	368	64
	10	10	381	77
stengel 1	1	0.66	319	15
	5	3.3	346	42
	10	6.6	354	50
stengel 2	1	0.75	323	19
	5	3.75	343	39
	10	7.5	374	69
stengel 4	1	0.64	320	16
	5	3.2	336	32
	10	6.4	468	64
bellen 1	1	0.61	325	21
	5	3.05	351	47
	10	6.1	365	61
bellen 2	1	0.65	316	12
	5	3.25	342	38
	10	6.5	366	62
bellen 3	1	0.56	319	15
	5	2.8	346	42
	10	5.6	360	56
bellen 4	1	0.6	313	9
	5	3	341	37
	10	6	359	55
afval 1	1	0.55	320	16
	5	2.75	---	--
	10	5.5	345	41
afval 2	1	0.56	320	16
	5	2.8	339	35
	10	5.6	351	47
afval 3	1	0.72	309	5
	5	3.8	334	30
	10	7.2	360	56
afval 4	1	0.65	314	10
	5	3.25	343	39
	10	6.5	352	48

BIJLAGE 5 RESULTATEN SCHUIMSTABILITEIT NA TOEVOEGING VAN PECTINEN AAN BIER (PECTINEN GEISOLEERD UIT DUITSE HOP) - toevoeging gebasseerd op gemeten zuiverheid (bijlage 8)

experiment [9-11-1993]	gehalte toegevoegd (g/hl)	werkelijk gehalte (AUA g/hl)	schuimstabiliteit sec)	proef t.o.v. blanco sec)
blanco water	--		101	--
stengel 1	5.1	5	122	21
	11	5	148	47
	21	10	159	58
stengel 4	4.7	5	123	22
	23	5	141	40
	47	10	173	72
mentol	1.8	1.8	111	10
	3	3	129	28
	9	9	143	42
	15	15	158	57
	18	18	159	58
	30	30	178	77

BIJLAGE 6 ZUIVERHEID VAN DE PECTINEFRACTIES (AUA-GEHALTE) EN DE VERESTERINGSGRAAD (DE) VAN DE DUITSE HOPVARIETEITEN

monster	AUA (mg)	AUA (%)	DE (%)
pectine commercieel	285	95	69
stengel 1	146	66	76
stengel 2	227	75	70
stengel 4	192	64	73
hoobel 1	183	61	69
hoobel 2	194	65	75
hoobel 3	164	56	72
hoobel 4	181	60	68
afval 1	164	55	75
afval 2	167	56	77
afval 3	215	72	70
afval 4	195	65	77

VOORBEELD 3

3.1 MATERIAAL

Er is gebruik gemaakt van residuen van de volgende hopextracten:

A Ethanol extract residuen
B CO_2 extract residuen
C CO_2 extract residuen
D Hexaan extract residuen

De schuimstabilisatie experimenten zijn ter vergelijking ook uitgevoerd met commerciële citruspectine (DE 67%), montol en eerder gezuiverde pectine fracties uit hop stengels en hop bellen (voorbeeld 2)

Voor de schuimexperimenten is referentie bier gebruikt.

3.2 METHODEN

3.2.1) Extractie van pectine

De gemalen residuen worden met water (aangezuurd tot pH 2) geëxtraheerd om pectine te isoleren. De gevolgde procedure is vermeld in bijlage 1.

3.2.2) Bepaling schuiminvloed pectine

De gezuiverde pectinefracties zijn aan bier toegevoegd om de invloed ervan op de schuimstabiliteit te bepalen. De procedure staat beschreven in bijlage 2.

3.3 RESULTATEN

Van verschillende hopleveranciers zijn residuen verkregen die overblijven na het vervaardigen van hopextracten. Uit deze residuen zijn pectines geïsoleerd. De opbrengsten zijn in tabel 5 weergegeven. De opbrengsten aan pectine uit deze residuen zijn vergelijkbaar met de opbrengsten uit vers materiaal (bellen en stengels). Residuen van CO_2 extracten waren van twee leveranciers afkomstig en geven een verschillende opbrengst aan pectine te zien. De extractie-procedure is echter niet volledig bekend voor beide leveranciers en er is gebruik gemaakt van verschillende hop

variëteiten. Uit voorbeeld 2 is gebleken dat de variëteit van invloed is op de hoeveelheid pectine die geïsoleerd kan worden.

Tabel 5 Opbrengst van pectinefracties gezuiverd uit residuen welke zijn onstaan bij de bereiding van verschillende hopextracten

Monster		Opbrengst pectine (%)
residuen ethanol extract	A	2,3
residuen CO_2 extract	B	1,8
residuen CO_2 extract	C	2,5
residuen hexaanextract	D	2,4

In figuur 8 is de invloed van de residu pectines weergegeven op de schuimstabiliteit van pilsner bier in vergelijking met commercieel citruspectine, montol en stengelpectine (zie voorbeeld 2). In bijlage 3 zijn de resultaten in tabellen weergegeven. Bij de dosering van de pectinefracties is uitgegaan van 100% zuiverheid. Het AUA-gehalte van de residufracties zal echter lager zijn (is niet bepaald). Bij de vergelijking met montol moet hiermee rekening gehouden worden.

De schuimstabiliserende werking van pectine uit ethanol- en hexaan extract residuen is niet noemenswaardig. Bier waaraan deze pectines zijn toegevoegd vertonen een vrijwel gelijke schuimstabilisatie als blanco bier. Een positief effect kan worden waargenomen na toevoeging van pectines uit residuen van CO_2 extracten. Bij een toevoeging van 10 g pectine/hl bedraagt de schuimverbetering 26 sec. De pectines uit stengel en bellen (vorig onderzoek) geven een verhoging van 40 sec, waarbij echter niet gecorrigeerd is voor de zuiverheid van de fracties.

BIJLAGE 1 (bij voorbeeld 3) **EXTRACTIE VAN PECTINE UIT HOP**

1 De residuen van de verschillende extracten malen (vooraf bevriezen met stikstof)
2 Warm water toevoegen (water:materiaal verhouding, zie tabel 4.1), aanzuren met HCl tot pH 2
3 Gedurende 2 uur bij 80°C onder constant roeren Filtreren over kaasdoek Supernatant mengen met alcohol 96% (1:1,5) zonder te neutraliseren
4 Filtreren over kaasdoek
5 Neerslag 3x uitwassen met 96% alcohol
6 Over kaasdoek filtreren
7 Gedurende de nacht drogen in petrischaal

BIJLAGE 2 (bij voorbeeld 3) **BEPALING SCHUIMINVLOED VAN PECTINES**

1 Gedroogde pectines worden fijngemaakt en onder verwarming opgelost in 5 ml water alvorens ze worden toegevoegd aan bier in de volgende concentraties: 15 mg/flesje (± 5 g/hl) en 30 mg/flesje (± 10 g/hl). Hierbij is ervan uitgegaan dat de pectinefracties 100% zuiver waren.
2 De flesjes worden geschud (50 rpm) gedurende 60 uur bij kamertemperatuur.
3 Meting van de schuimstabiliteit met de Nibem-meter.

BIJLAGE 3 SCHUIMSTABILITEIT VAN BIEREN WAARAAN VERSCHILLENDE PECTINEFRACTIES ZIJN TOEGEVOEGD
(bij voorbeeld 3)

Monster	Toegevoegde hoeveelheid g/hl	Schuim-stabiliteit sec	Verhoogde stabiliteit sec
Blanco	--	280	--
Blanco water	--	300	--
Residuen hexaan	5	300	0
extract A	10	294	0
Residuen CO_2	5	302	2
extract B	10	327	27
Residuen CO_2	5	300	0
extract C	10	326	26
Residuen ethanol	5	296	0
extract D	10	298	0
Montol	5	345	45
	10	361	61
Commercieel	5	323	23
pectine	10	355	55
Stengel 1	10	344	44
Bellen 1	10	338	38

CONCLUSIES

1. Werkwijze voor het verbeteren van de stabiliteit van de schuimkraag van dranken waarbij aan de drank voor, tijdens of na het bereidingsproces daarvan één of meer pectines worden toegevoegd.

2. Werkwijze volgens conclusie 1, waarbij een uit hop verkregen extract van één of meer pectines wordt toegepast.

3. Werkwijze volgens conclusie 2, waarbij het extract wordt verkregen uit stengels en/of bellen van de hopplant.

4. Werkwijze volgens conclusie 1-3, waarbij de drank bier is.

5. Werkwijze volgens conclusie 4, waarbij pectines worden toegevoegd tijdens het bereidingsproces vanaf 30 minuten voor einde wortkoken, zodanig dat er geen significant deel van de schuim-stabiliserende activiteit verloren gaat door het koken.

6. Werkwijze volgens conclusie 5, waarbij pectines worden toegevoegd voor de helderbierfiltratie.

7. Werkwijze volgens één der conclusies 4-6, waarbij tussen 0,5 en 30 g pectine per hectoliter bier wordt toegevoegd.

8. Werkwijze volgens conclusie 7, waarbij ongeveer 3-10 g pectine per hectoliter bier wordt toegevoegd.

9. Drank met een gestabiliseerde schuimkraag verkrijgbaar met een werkwijze volgens één der voorgaande conclusies.

10. Bier met een gestabiliseerde schuimkraag verkrijgbaar met een werkwijze volgens één der conclusies 1-8.

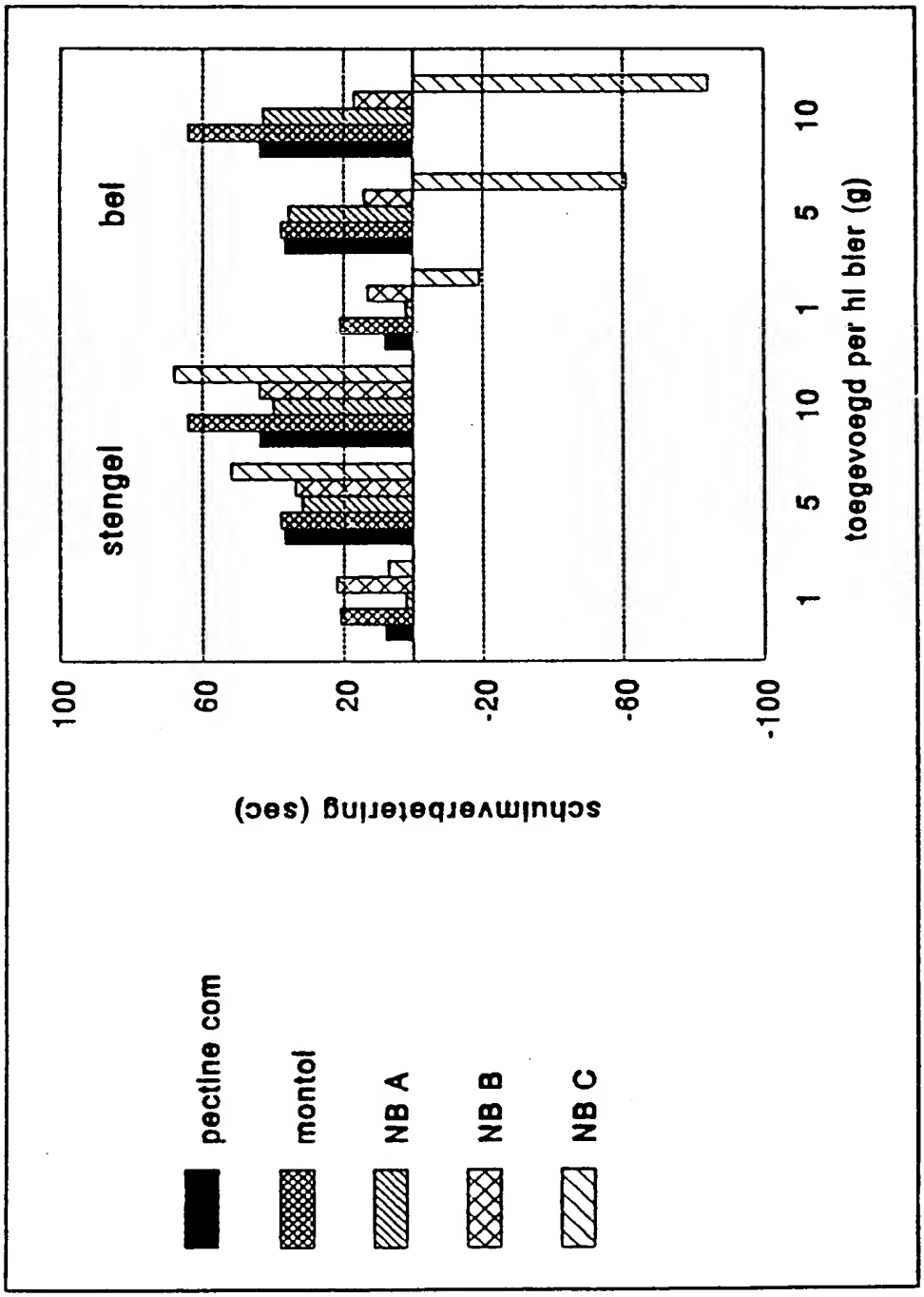
11. Bier verkregen volgens één der conclusies 1-8.

12. Het gebruik van hoppectines als schuimstabilisator voor schuimkragen van dranken.

13. Werkwijze voor het extraheren van pectines uit hop, waarbij hopplanten of delen daarvan worden onderworpen aan een extractie in een waterige oplossing bij een temperatuur van 50-100°C en een pH van 1-3,5.

UITTREKSEL

De uitvinding voorziet in pectines als nieuwe schuimstabilisatoren voor (tijdelijke) schuimkragen bij dranken, met name bij bieren, in het bijzonder bieren van het pilsner type. Deze schuimstabilisatoren worden bij voorkeur verkregen uit hop, hetgeen een bier-eigen bestanddeel is en daarmee onder andere het voordeel biedt dat de schuimstabilisatoren geen negatieve invloed op de smaak van het bier behoeven te hebben. Bij voorkeur worden de pectines verkregen uit hopbellen of stengels. Het kan voordelig zijn om de pectines gedeeltelijk te verzepen, in verband met het aantal geladen groepen aan de pectines. Daarnaast voorziet de uitvinding in werkwijzen voor het verkrijgen van de pectines en dranken gestabiliseerd met de pectines volgens de uitvinding.



Verbetering van de schuimstabiliteit van pilsner referentiebier, na toevoeging van hoppectine (uit stengels of bellen), commercieel pectine (100%) en montol (100%)

FIG.1

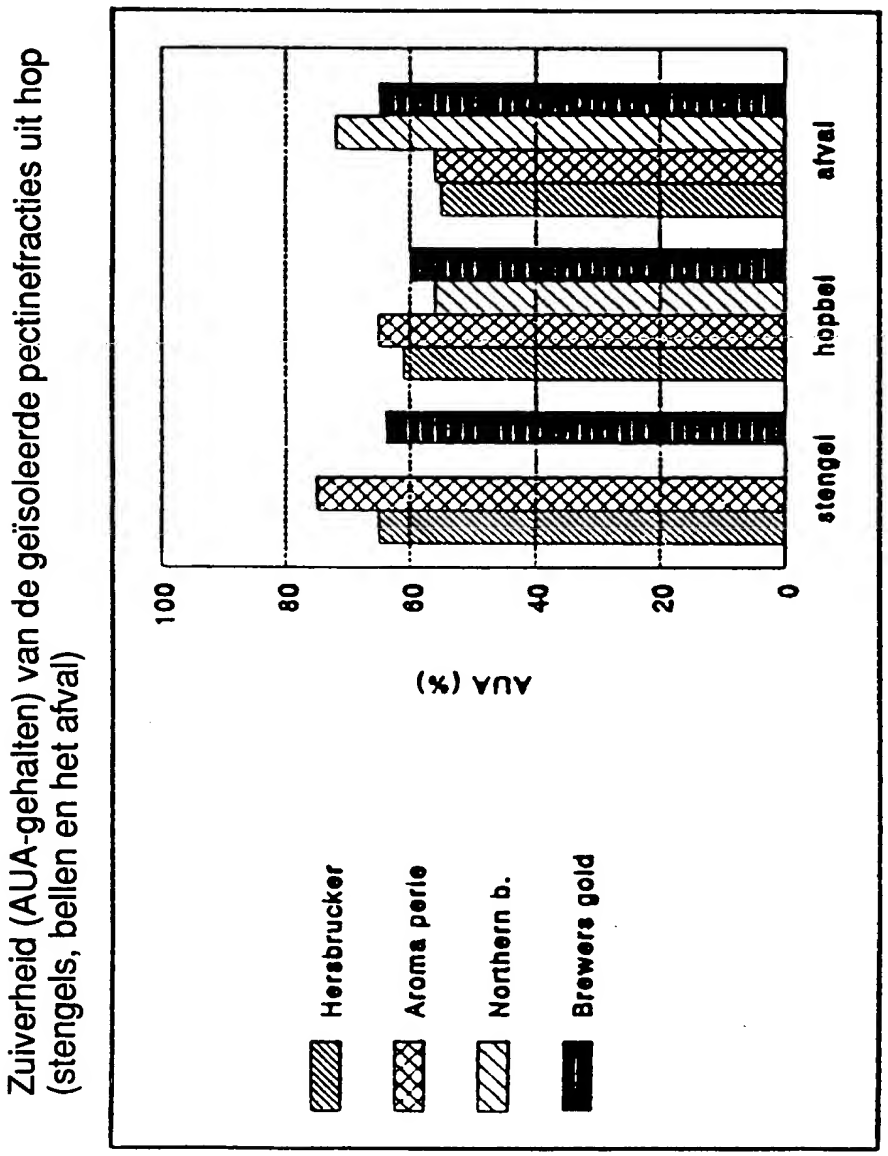

Zuiverheid (AUA-gehalten) van de geïsoleerde pectinefracties uit hop
(stengels, bellen en het afval)
AUA (%)
100
80
60
40
20
0
stengel
hopbel
afval
Hersbrucker
Aroma perle
Northern b.
Brewers gold

FIG.2

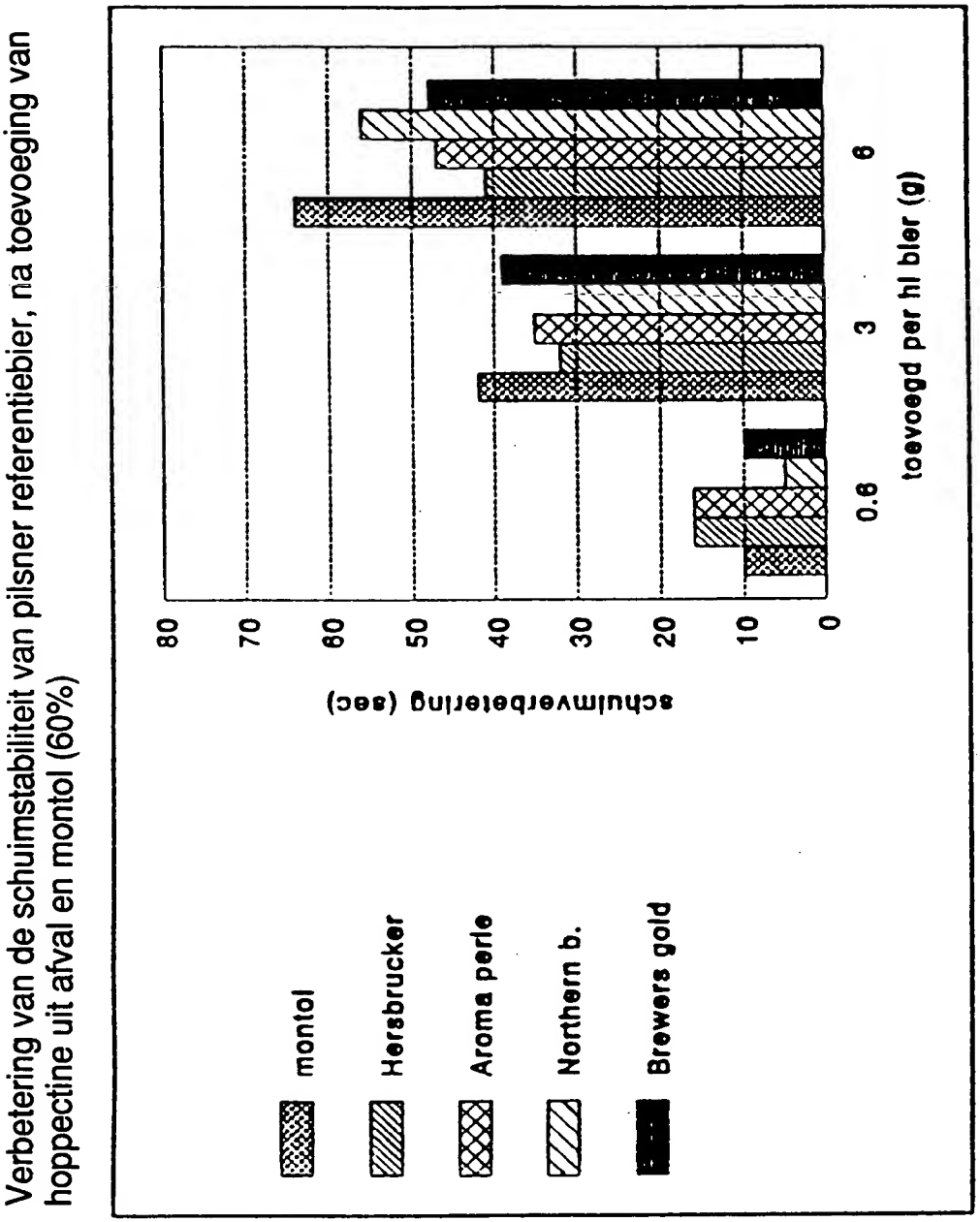

Verbetering van de schuimstabiliteit van pilsner referentiebier, na toevoeging van hoppectine uit afval en montol (60%)
schuimverbetering (sec)
0
10
20
30
40
50
60
70
80
0.6
3
6
toevoegd per hl bier (g)
montol
Hersbrucker
Aroma perle
Northern b.
Brewers gold

FIG.3

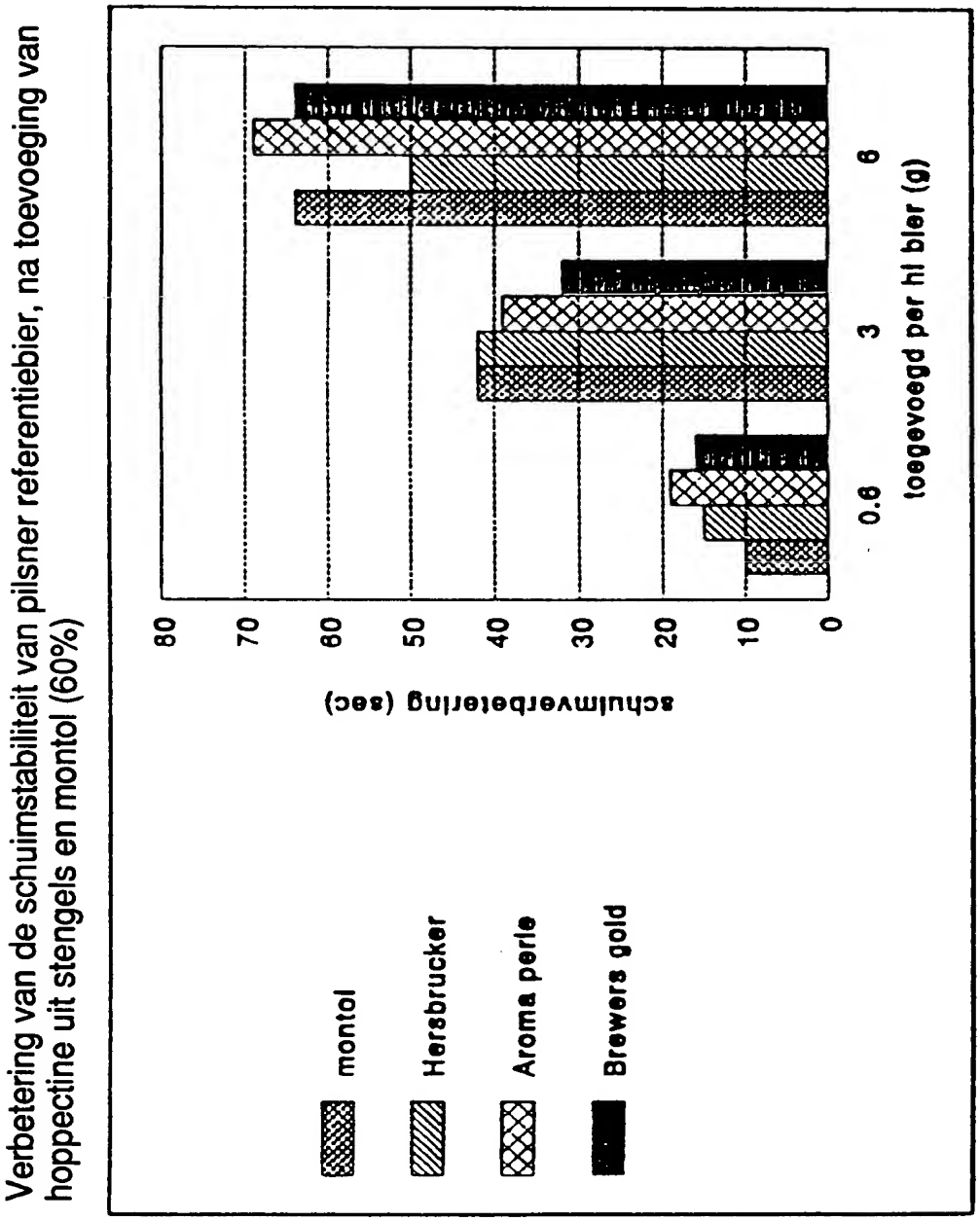

Verbetering van de schuimstabiliteit van pilsner referentiebier, na toevoeging van hoppectine uit stengels en montol (60%)
schuimverbetering (sec)
80
70
60
50
40
30
20
10
0
0.6
3
6
toegevoegd per hl bier (g)
montol
Hersbrucker
Aroma perle
Brewers gold

FIG.4

Verbetering van de schuimstabiliteit van (pilsner) referentiebier, na toevoeging van hoppectine uit bellen en montol (60%)

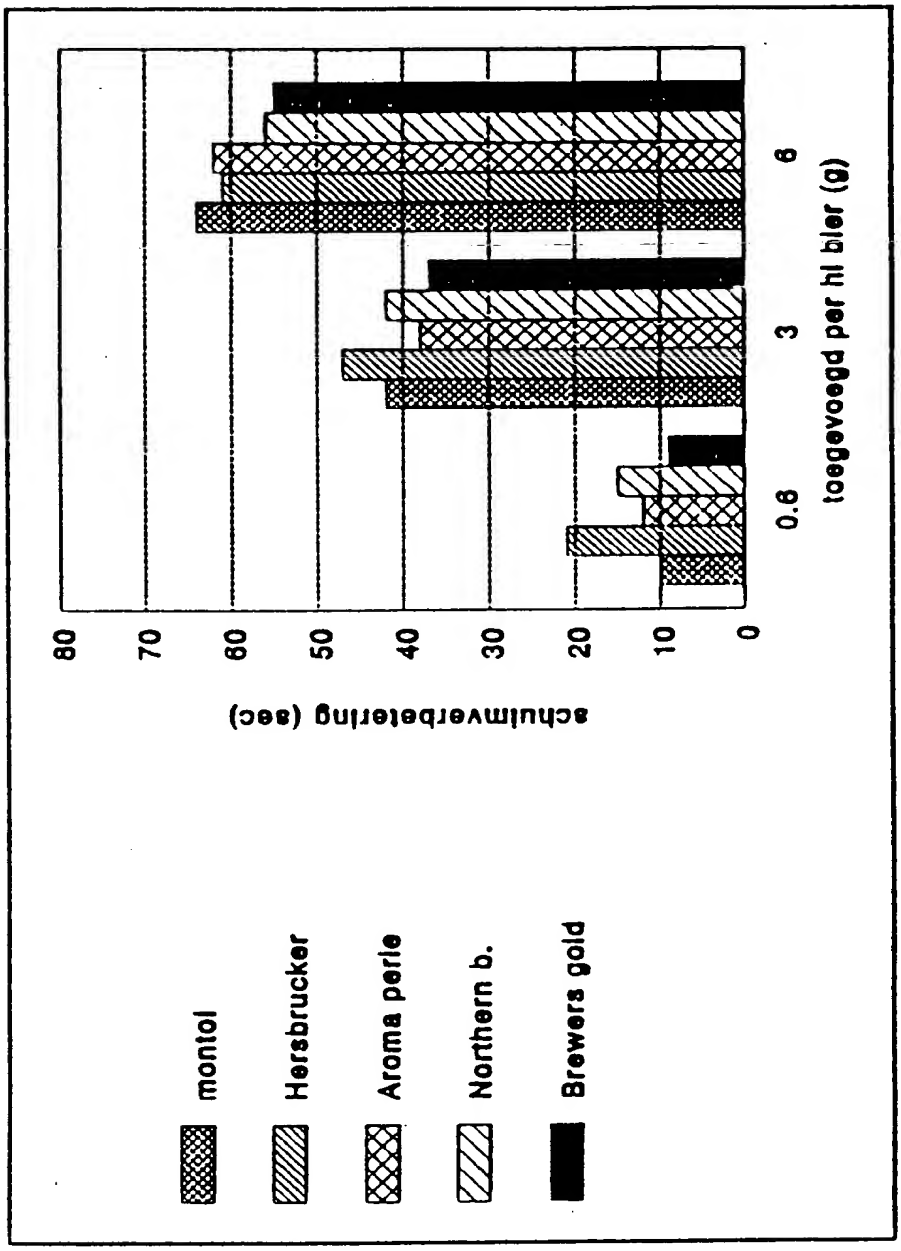


FIG.5

Verbetering van de schuimstabiliteit van pilsner referentiebier, na toevoeging van hoppectine uit stengels, (gecorrigeerd) en montol (100%)

Verbetering van de schuimstabiliteit van referentiebier, na toevoeging van hoppectine uit stengels, (gecorrigeerd) en montol (100%)

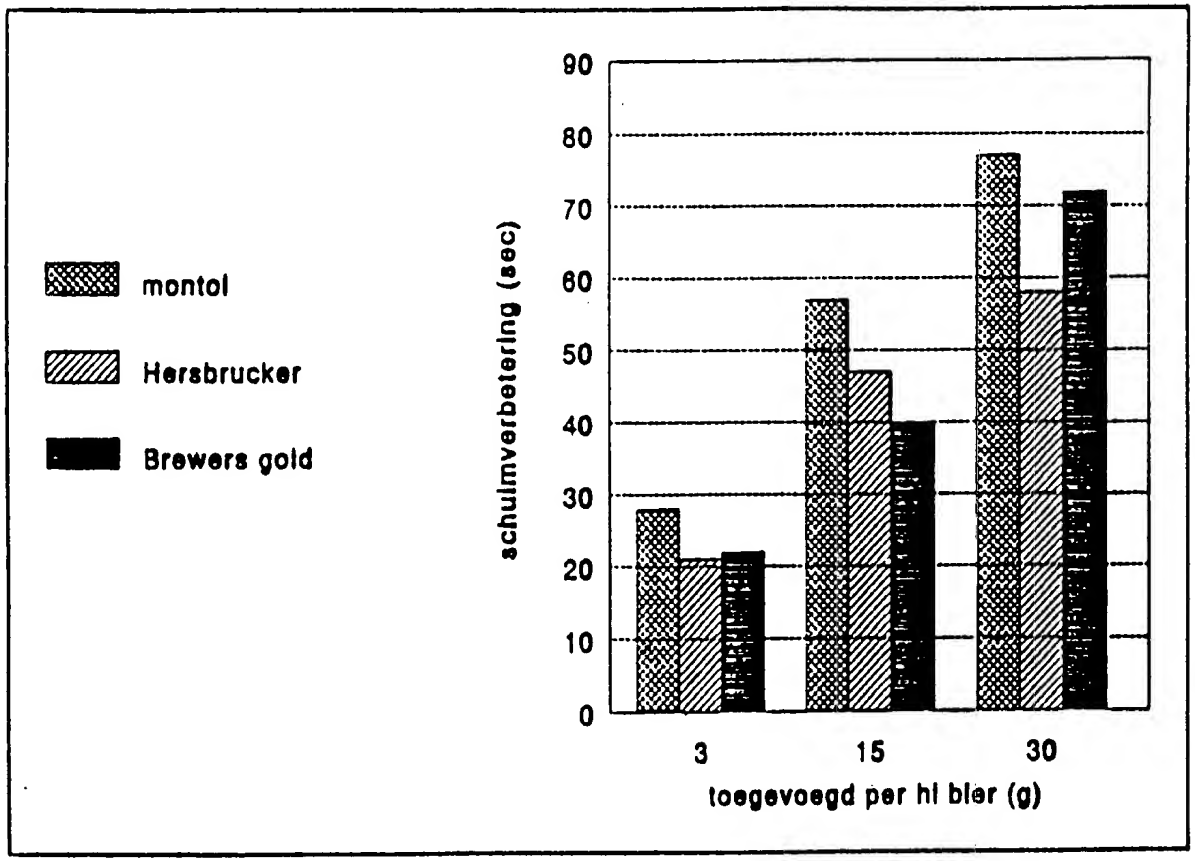


FIG.6

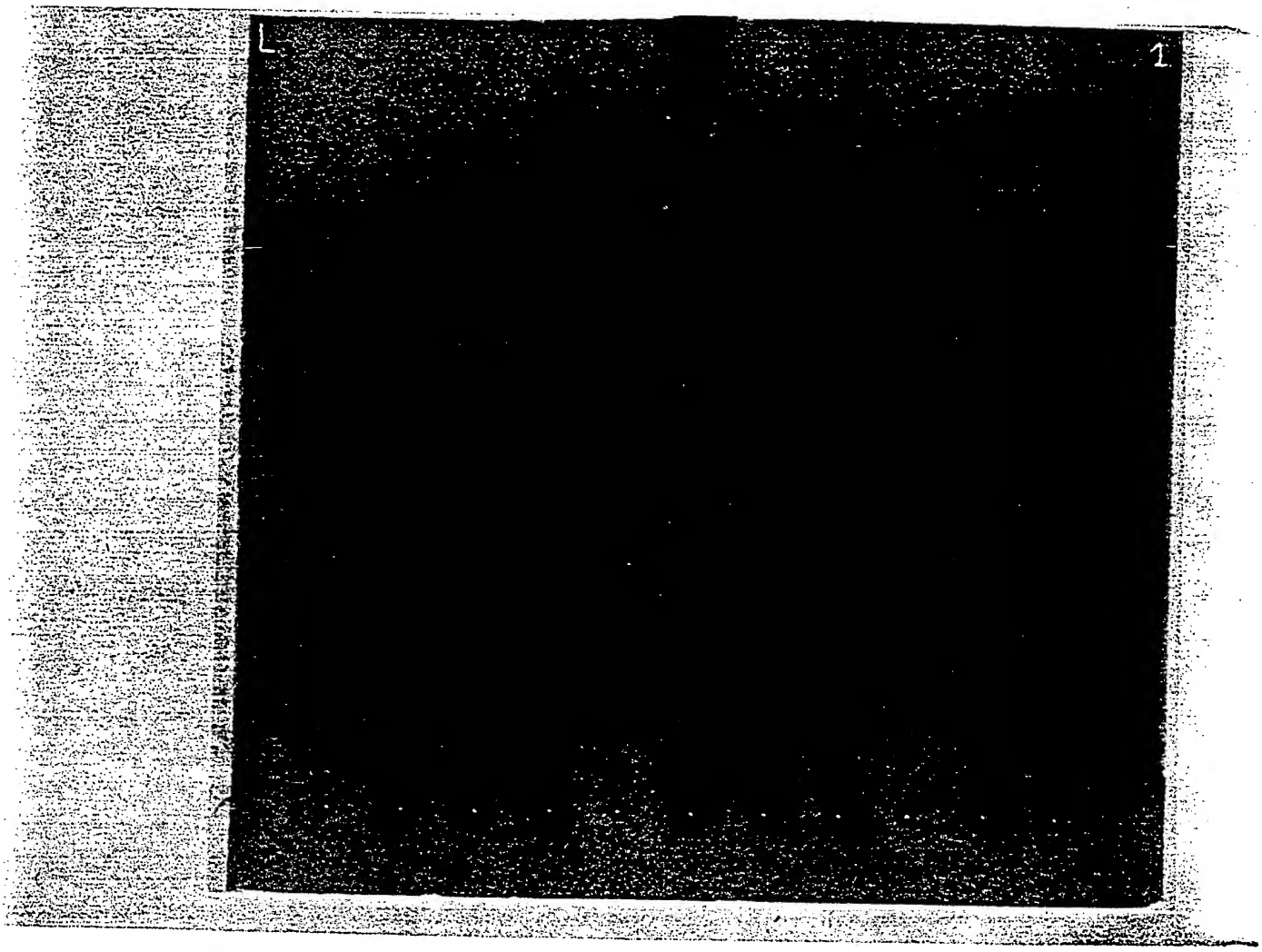


Fig. 7-1

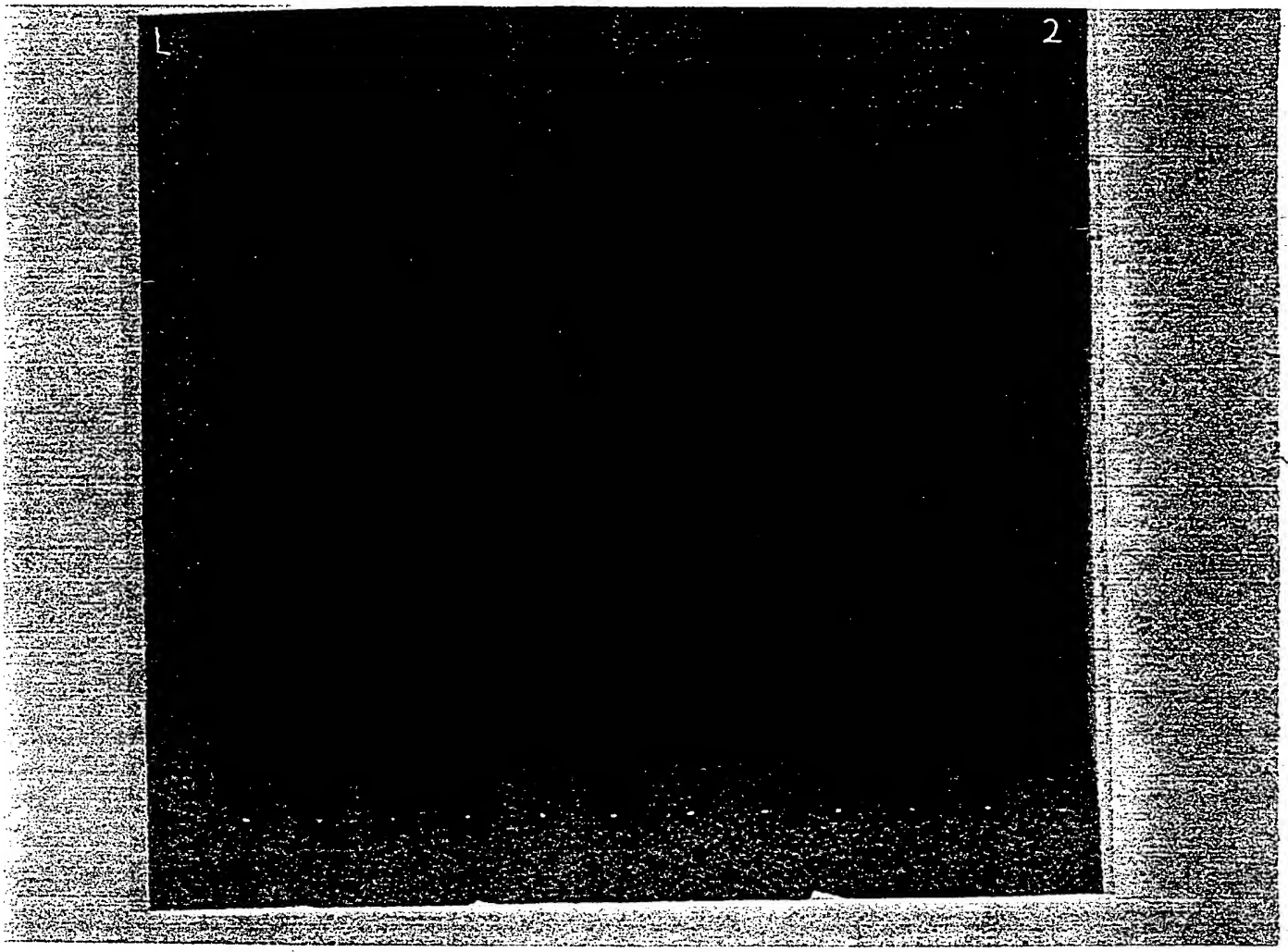


Fig. 7-2

Verbetering van de schuimstabiliteit van pilsner referentiebier, na toevoeging van hoppectine uit residuen van hexaan-, ethanol- en CO 2-extracten, montol (100%), stengel pectine, hopbel pectine en commercieel pectine (100%)

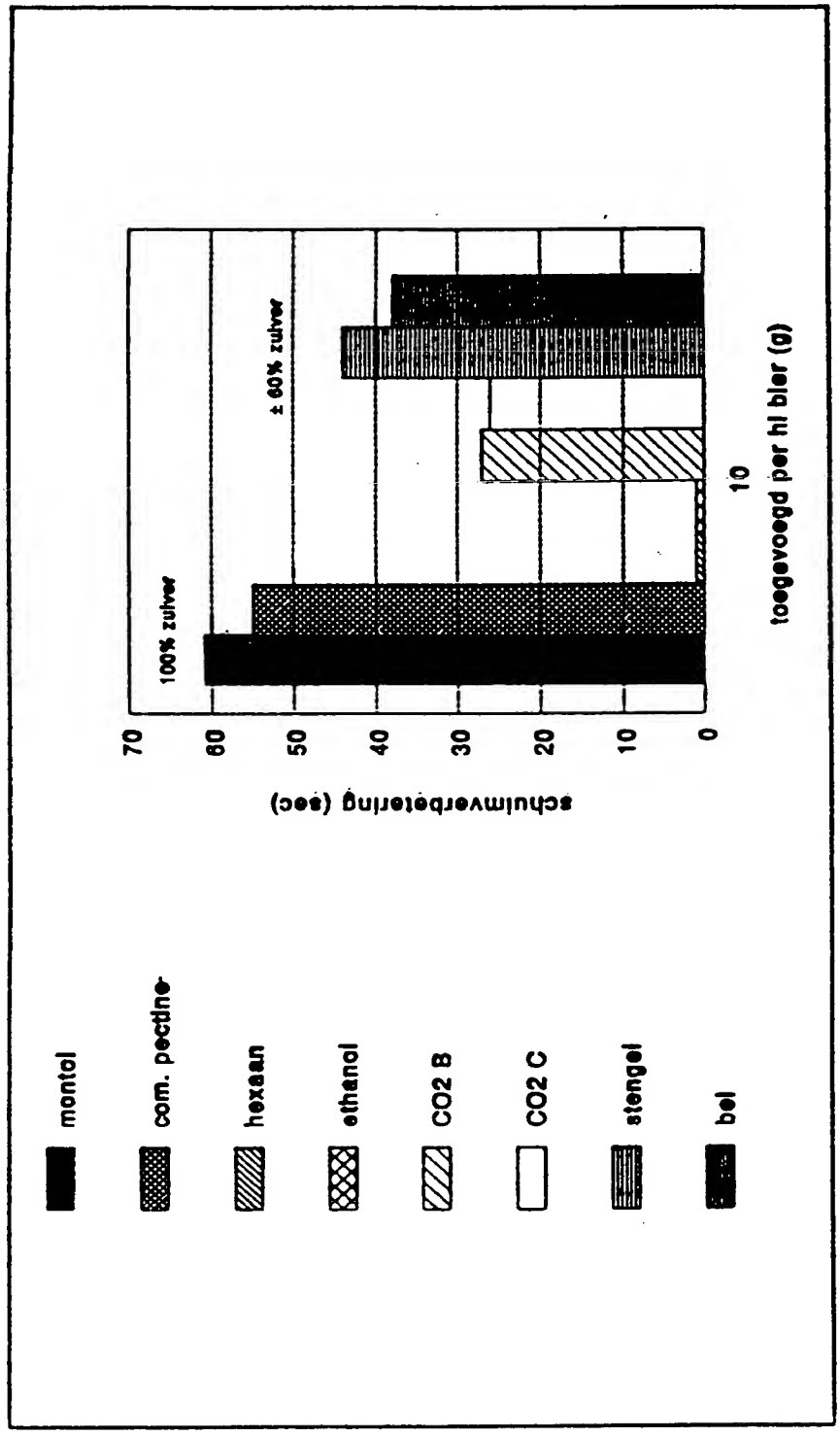


FIG.8

PATENT COOPERATION TR TY

PCT

01 OCT. 1996

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PCT 0396		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL 95/ 00266	03/08/1995	04/08/1994
International Patent Classification (IPC) or national classification and IPC		
C12H1/14		
Applicant		
HEINEKEN TECHNICAL SERVICES B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consists of a total of _____ sheets.

3. This report contains indications and corresponding pages relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/02/1996	27.09.96
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	M. Wieser Telephone No. 2399-8434

Intern. application No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

PCT/NL95/00266

I. Basis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

[x] the international application as originally filed.

[ ] the description, pages _____, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,
pages _____, filed with the letter of _____,

[ ] the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. _____, filed with the letter of _____,
Nos. _____, filed with the letter of _____,

[ ] the drawings, sheets/fig _____, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[ ] the description, pages _____.
[ ] the claims, Nos. _____.
[ ] the drawings, sheets/fig _____.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

[ ] restricted the claims.
[ ] paid additional fees.
[ ] paid additional fees under protest.
[ ] neither restricted nor paid additional fees.

2. [x] This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
[ ] complied with.
[x] not complied with for the following reasons:

Claims 1-12 refer to a method for improving the foam stability of beverages, preferably beer, by addition of pectins, preferably obtained from hops, and to the use of hop pectins as foam stabilizers.

Claim 13 refers to a process for extracting pectins from hops.

These separate inventions are not so linked as to form a single general inventive concept.

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

[x] all parts.
[ ] the parts relating to claims Nos. _____.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 2,3,12,13	YES
	Claims 1,4-11	NO
Inventive Step (IS)	Claims	YES
	Claims 1-13	NO
Industrial Applicability (IA)	Claims 1-13	YES
	Claims	NO

2. CITATIONS AND EXPLANATIONS

The following documents mentioned in the International Search Report are considered as being the most relevant prior art:

(A) DATABASE FSTA
INT. FOOD INFORMATION SERVICE
AN 00188037, July 1980
& SU-A-685 689

(B) EP-A-0 292 034

(C) DATABASE FSTA
INT. FOOD INFORMATION SERVICE
AN 00089682, Dec. 1974
& Izvestiya Vysshikh Uchebnykh Zavedenii
1974, no.1,pp21-25

(D) GB-A-1 048 912

1. Document A (see abstract) discloses the subject-matter of claims 1 and 4-11. The subject of claims 1 and 9 is moreover disclosed in document B (see examples and

claims).

Therefore, the subject-matter of claims 1 and 4-11 is not novel and does not meet the requirements of Article 33(2) PCT.

2. The subject-matter of claims 2,3,12 and 13 is distinguished from the disclosure in documents A and B by the fact that the used pectins are obtained from hops. According to page 2 of the description, these pectins do not affect the taste properties of beer since they are obtained from an ingredient that is inherent in beer.

The problem to be solved by the invention was therefore to replace foam stabilizers used in the prior art by substances which are derived from ingredients that are inherent in beer.

Document C discloses that hops contain 2% of pectic substances, which are surface active and a foaming agent. Document D discloses the use of a hop extract containing 70-80% of bitter principles and 20-30% of a mixture of tannin, pectin, albumen, cellulose, aromatic acids and water for the production of beer and describes process for obtaining said extract.

A skilled person being aware of this state of the art and of the problem to be solved, would arrive at the subject-matter of claims 1-13 without being inventive.

Therefore, claims 1-13 do not meet the requirements of Article 33(3) PCT.

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

1. The documents A-D have not been identified in the description nor has the relevant background art disclosed therein been discussed. The requirements of Rule 5.1(a)(ii) PCT are, thus, not fulfilled.

2. Terms, used in the description, which are registered trade marks have not been identified as such.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

For the assessment of the present claims 9-11 on the question whether they are novel, no unified criteria exist in the PCT. The EPO, for example, does not recognize novel the subject-matter of claims to products defined by a process for their manufacture, when the product as such is not novel.

PATENT COOPERATION TR[illegible]Y

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
PCT 0396		
International application No.	International filing date (day/month/year)	(Earliest) Priority Date (day/month/year)
PCT/NL95/00266	03/08/95	04/08/94
Applicant		
HEINEKEN TECHNICAL SERVICES B.V. et al.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. 1
   - [X] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 95/00266

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12H1/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12H C12C A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 292 034 (EVERS PAULUS HENDRICUS JOHANNN) 23 November 1988 see page 2, line 15 - line 17 ---	1,9,11
X	DATABASE FSTA INTERNATIONAL FOOD INFORMATION SERVICE (IFIS), FRANFURT/MAIN, DE AN 00188037, July 1980 SHKOP, YA. F. ET AL '(Method of producing beer.)' see abstract & SU,A,685 689 1979 ---	1,4,9-11
X	EP,A,0 426 434 (SBP, INC.) 8 May 1991 cited in the application see claims; example 2	1,9
A	---	11-13
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search
19 October 1995

Date of mailing of the international search report
29. 11. 95

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer
Bevan, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 463 696 (DE MELKINDUSTRIE VEGHEL B.V.) 2 January 1992 see the whole document	1,4,11
Y	GB,A,1 048 912 (M. BRIEM) 23 November 1966 see the whole document	1-13
Y	WO,A,93 15181 (RHONE-POULENC, INC.) 5 August 1993 see claims	1-13
A	DATABASE FSTA INTERNATIONAL FOOD INFORMATION SERVICE (IFIS), FRANFURT/MAIN, DE AN 00089682, December 1974 DROZDOVA, G. G. ET AL '(Pectic substances in barley and their role in beer and malt production.)' see abstract & IZVESTIYA VYSSHIKH UCHEBNYKH ZAVEDENII, PISHCHEVAYA TEKHNOLOGIYA, 1974, NO. 1, 21-25,	1,4,11
A	US,A,4 808 574 (I.I. BREKHMAN ET AL.) 28 February 1989 see column 2, line 13 - line 45	1,4,11
A	EP,A,0 243 654 (PEKTIN-FABRIK HERMANN HERBSTREITH KG) 4 November 1987 cited in the application see examples	1
A	US,A,3 099 563 (P.L.SMITH) 30 July 1963 cited in the application	
A	GB,A,1 082 284 (C. GORTATOWSKY) 6 September 1967	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 95/00266

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0292034	23-11-88	NL-A-	8700955	16-11-88
		CA-A-	1297820	24-03-92
		DE-A-	3872049	23-07-92
		US-A-	4943443	24-07-90
EP-A-426434	08-05-91	US-A-	5008254	16-04-91
		AU-B-	630755	05-11-92
		AU-B-	6564190	09-05-91
		CA-A-	2029022	02-05-91
		JP-A-	3197502	28-08-91
EP-A-463696	02-01-92	NL-A-	9001438	16-01-92
GB-A-1048912		NONE		
WO-A-9315181	05-08-93	AU-B-	3606393	01-09-93
		CA-A-	2127440	05-08-93
		CN-A-	1082104	16-02-94
		EP-A-	0625188	23-11-94
		FI-A-	943599	02-08-94
		NO-A-	942868	30-09-94
		US-A-	5387425	07-02-95
US-A-4808574	28-02-89	WO-A-	8911284	30-11-89
EP-A-243654	04-11-87	DE-C-	3614656	25-06-87
		AU-B-	7141487	05-11-87
		JP-A-	63039564	20-02-88
		ZA-A-	8703041	21-10-87
US-A-3099563	30-07-63	NONE		
GB-A-1082284		NONE		